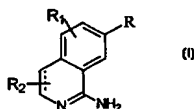




INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : C07K 5/06, 5/08, A61K 38/05, 38/06, 38/07, 31/47, C07D 217/22, 401/12, 401/14, 405/12, 409/14 // (C07D 401/12, 217:00, 207:00) (C07D 401/12, 217:00, 211:00)	A1	(11) International Publication Number: WO 99/11657 (43) International Publication Date: 11 March 1999 (11.03.99)
(21) International Application Number: PCT/GB98/02600 (22) International Filing Date: 28 August 1998 (28.08.98) (30) Priority Data: 9718392.5 29 August 1997 (29.08.97) GB 9803173.5 13 February 1998 (13.02.98) GB (71) Applicant (for all designated States except US): PROTEUS MOLECULAR DESIGN LTD. [GB/GB]; Beechfield House, Lyme Green Business Park, Macclesfield, Cheshire SK11 0JL (GB). (72) Inventors; and (75) Inventors/Applicants (for US only): LIEBESCHUETZ, John, Walter [GB/GB]; Beechfield House, Lyme Green Business Park, Macclesfield, Cheshire SK11 0JL (GB). WYLIE, William, Alexander [GB/GB]; Beechfield House, Lyme Green Business Park, Macclesfield, Cheshire SK11 0JL (GB). WASZKOWYCZ, Bohdan [GB/GB]; Beechfield House, Lyme Green Business Park, Macclesfield, Cheshire SK11 0JL (GB). MURRAY, Christopher, William [GB/GB]; Beechfield House, Lyme Green Business Park,		Macclesfield, Cheshire SK11 0JL (GB). RIMMER, Andrew, David [GB/GB]; Beechfield House, Lyme Green Business Park, Macclesfield, Cheshire SK11 0JL (GB). WELSH, Pauline, Mary [GB/GB]; Beechfield House, Lyme Green Business Park, Macclesfield, Cheshire SK11 0JL (GB). JONES, Stuart, Donald [GB/GB]; Beechfield House, Lyme Green Business Park, Macclesfield, Cheshire SK11 0JL (GB). ROSCOE, Jonathan, Michael, Ernest [GB/GB]; Beechfield House, Lyme Green Business Park, Macclesfield, Cheshire SK11 0JL (GB). YOUNG, Stephen, Clinton [GB/GB]; Beechfield House, Lyme Green Business Park, Macclesfield, Cheshire SK11 0JL (GB). MORGAN, Phillip, John [GB/GB]; Beechfield House, Lyme Green Business Park, Macclesfield, Cheshire SK11 0JL (GB). CAMP, Nicholas, Paul [GB/GB]; Beechfield House, Lyme Green Business Park, Macclesfield, Cheshire SK11 0JL (GB). CREW, Andrew, Philip, Austin [GB/GB]; Beechfield House, Lyme Green Business Park, Macclesfield, Cheshire SK11 0JL (GB). (74) Agents: COCKBAIN, Julian et al.; Frank B. Dehn & Co., 179 Queen Victoria Street, London EC4V 4EL (GB). (81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i>

(54) Title: 1-AMINO-7-ISOQUINOLINE DERIVATIVES AS SERINE PROTEASE INHIBITORS



(57) Abstract

The invention relates to serine protease inhibitor compounds of formula (I) where R₁ is hydrogen, halo, cyano, nitro or hydroxyl, amino, alkoxy, alkyl, aminoalkyl, hydroxyalkyl, thiol, alkylthio, aminosulphonyl, alkoxyalkyl, alkoxyacetyl, acyloxymethoxycarbonyl or alkylamino optionally substituted by hydroxy, alkylamino, alkoxy, oxo, aryl, cycloalkyl, amino, halo, cyano, nitro, thiol, alkylthio, alkylsulphonyl, alkylsulphenyl, alkylsulphonamido, alkylaminosulphonyl, haloalkoxy and haloalkyl; R₂ is hydrogen, halo, methyl, amino, hydroxy, or oxo; and R is X-X-Y(R₇)-L-Lp(D)n; wherein each X independently is a C, N, O or S atom or a CO, CR₁, C(R₁)₂ or NR₁ group, at least one X being C, CO, CR₁ or a C(R₁)₂ group; Y (the α-atom) is a nitrogen atom or a CR₁ group or Y and L taken together form a cyclic group; R₇ is a lipophilic group selected from alkyl, alkenyl, mono- or bi-cycloalkyl, aryl, heteroaryl, mono- or bicycloalkylalkyl, mono- or bicycloalkylalkenyl, aralkyl, heteroaryl-alkyl, arylalkenyl, heteroarylalkenyl all optionally substituted by a group R₁; L is an organic linker group containing 1 to 5 backbone atoms selected from C, N, O and S, or a branched alkyl or cyclic group; Lp is a lipophilic organic group selected from alkyl, heterocyclic, alkenyl, alkaryl, cycloalkyl, polycycloalkyl, cycloalkenyl, aryl, aralkyl or haloalkyl group or a combination of two or more such groups optionally substituted by one or more of oxa, thia, aza or R₁ groups; D is a hydrogen bond donor group; and n is 0, 1 or 2 and salts thereof.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece			TR	Turkey
BG	Bulgaria	HU	Hungary	ML	Mali	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MN	Mongolia	UA	Ukraine
BR	Brazil	IL	Israel	MR	Mauritania	UG	Uganda
BY	Belarus	IS	Iceland	MW	Malawi	US	United States of America
CA	Canada	IT	Italy	MX	Mexico	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NE	Niger	VN	Viet Nam
CG	Congo	KE	Kenya	NL	Netherlands	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NO	Norway	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	NZ	New Zealand		
CM	Cameroon	KR	Republic of Korea	PL	Poland		
CN	China	KZ	Kazakhstan	PT	Portugal		
CU	Cuba	LC	Saint Lucia	RO	Romania		
CZ	Czech Republic	LI	Liechtenstein	RU	Russian Federation		
DE	Germany	LK	Sri Lanka	SD	Sudan		
DK	Denmark	LR	Liberia	SE	Sweden		
EE	Estonia			SG	Singapore		

1-AMINO-7-ISOQUINOLINE DERIVATIVES AS SERINE PROTEASE INHIBITORS

5 This invention relates to compounds which are inhibitors of serine proteases and to pharmaceutical compositions thereof and their use in the treatment of the human or animal body.

10 The serine proteases are a group of proteolytic enzymes which have a common catalytic mechanism characterized by a particularly reactive Ser residue. Examples of serine proteases include trypsin, tryptase, chymotrypsin, elastase, thrombin, plasmin, kallikrein, Complement C1, acrosomal protease, lysosomal protease, cocoonase, α -lytic protease, protease A, protease B, 15 serine carboxypeptidase II, subtilisin, urokinase, Factor VIIa, Factor IXa, and Factor Xa. The serine proteases have been investigated extensively over a period of several decades and the therapeutic value of inhibitors of serine proteases is well understood.

20 Serine protease inhibitors play a central role in the regulation of a wide variety of physiological process including coagulation, fibrinolysis, fertilization, development, malignancy, neuromuscular patterning and inflammation. It is well known that 25 these compounds inhibit a variety of circulating proteases as well as proteases that are activated or released in tissue. It is also becoming clear that serine protease inhibitors inhibit critical cellular processes, such as adhesion, migration, free radical 30 production and apoptosis. In addition, animal experiments indicate that intravenously administered serine protease inhibitors, variants or cells expressing serine protease inhibitors, provide a protective effect against tissue damage.

35 Serine protease inhibitors have also been predicted to have potential beneficial uses in the treatment of disease in a wide variety of clinical areas such as

SUBSTITUTE SHEET (RULE 26)

- 2 -

oncology, neurology, haematology, pulmonary medicine, immunology, inflammation and infectious disease.

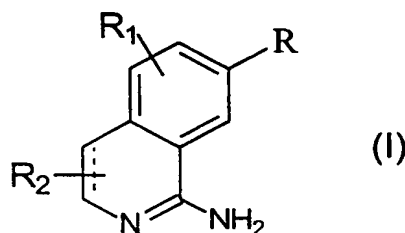
In particular serine protease inhibitors may be beneficial in the treatment of thrombotic diseases, asthma, emphysema, cirrhosis, arthritis, carcinoma, melanoma, restenosis, atheroma, trauma, shock and reperfusion injury.

Thus for example an inhibitor of Factor Xa has value as a therapeutic agent as an anticoagulant, e.g. in the treatment and prevention of thrombotic disorders. The use of a Factor Xa inhibitor as an anticoagulant is desirable in view of the selectivity of its effect. Many clinically approved anticoagulants have been associated with adverse events owing to the non-specific nature of their effects on the coagulation cascade.

Also, there are well-known associations of α_1 protease inhibitor deficiency with emphysema and cirrhosis and C1 esterase inhibitor deficiency with angioedema.

We have now found that certain novel amino substituted fused bicyclic compounds are particularly effective as inhibitors of serine proteases, especially proteases with negatively charged P1 specificity pockets, and most especially the serine proteases thrombin, trypsin, urokinase and Factor Xa. It is envisaged that compounds of the type described below will be readily bioavailable, particularly orally bioavailable.

Thus viewed from one aspect the invention provides serine protease inhibitor compounds of formula I



- 3 -

(where R_1 is hydrogen, halo, cyano, nitro or hydroxyl, amino, alkoxy, alkyl, aminoalkyl, hydroxyalkyl, thiol, alkylthio, aminosulphonyl, alkoxyalkyl, alkoxycarbonyl, acyloxymethoxycarbonyl or alkylamino optionally substituted by hydroxy, alkylamino, alkoxy, oxo, aryl, cycloalkyl, amino, halo, cyano, nitro, thiol, alkylthio, alkylsulphonyl, alkylsulphenyl, alkylsulphonamido, alkylaminosulphonyl, haloalkoxy and haloalkyl;

5 R_2 is hydrogen, halo, methyl, amino, hydroxy or oxo;
10 and

R is $X-X-Y(R_7)-L-Lp(D)_n$

(wherein each X independently is a C, N, O or S atom or a CO, CR_1 , $C(R_1)_2$ or NR_1 group, at least one X being C,
15 CO, CR_1 or a $C(R_1)_2$ group;

Y (the α -atom) is a nitrogen atom or a CR_1 group or Y and L taken together form a cyclic group;

R_7 is a lipophilic group, e.g. alkyl, alkenyl, mono- or bi-cycloalkyl, aryl, heteroaryl, mono- or
20 bicycloalkylalkyl, mono- or bicycloalkylalkenyl, aralkyl, heteroaryl-alkyl, arylalkenyl, heteroarylalkenyl all optionally substituted by a group R_1 ;

L is an organic linker group containing 1 to 5
25 backbone atoms selected from C, N, O and S, or a branched alkyl or cyclic group;

Lp is a lipophilic organic group, e.g. an alkyl, heterocyclic, alkenyl, alkaryl, cycloalkyl, polycycloalkyl, cycloalkenyl, aryl, aralkyl or haloalkyl
30 group or a combination of two or more such groups optionally substituted by one or more of oxa, thia, aza or R_1 groups, preferably a group containing up to 25 carbon atoms;

D is a hydrogen bond donor group; and n is 0, 1 or
35 2);

or a physiologically tolerable salt thereof, e.g. a halide, phosphate or sulphate salt or a salt with

ammonium or an organic amine such as ethylamine or meglumine.

In the compounds of the invention, unless otherwise indicated, aryl groups preferably contain 5 to 10 ring atoms optionally including 1, 2 or 3 heteroatoms selected from O, N and S; alkyl, alkenyl or alkynyl groups or alkylene moieties preferably contain up to 6 carbons; cyclic groups preferably have ring sizes of 3 to 8 atoms; and fused multicyclic groups preferably contain 8 to 16 ring atoms.

In the compounds of the invention the fused ring system is preferably a 1-aminoisoquinoline system.

Particularly as a substituent on the isoquinoline ring, R_1 is preferably hydrogen, hydroxy, amino or alkyl. R_2 is preferably hydrogen. Two or more non-hydrogen R_1 (or R_2) groups may be present on the carbocyclic (or heterocyclic) rings; however a single non-hydrogen R_1 (or R_2) group is preferred. R_1 is preferably on the 6-position of the fused ring system.

R is depicted in formula (I) as being present on the 7-position of the fused ring system although it is also envisaged that the lipophilic group could be present on the 6-position. However, preferably the lipophilic group will be present on the 7-position of the ring.

In the compounds of the invention, where the alpha atom (Y) is carbon it preferably has the conformation that would result from construction from a D- α -aminoacid $\text{NH}_2\text{-CR}_1(\text{R}_7)\text{-COOH}$. Likewise the fourth substituent R_1 at an alpha carbon is preferably a methyl or hydroxymethyl group or most preferably hydrogen.

The linker group X-X from the fused bicyclic group to the alpha atom is preferably selected from -CH=CH- , -CONH- , $\text{-CONR}_1\text{-}$, -NH-CO- , $\text{-NH-CH}_2\text{-}$, $\text{-CH}_2\text{-NH-}$, $\text{-CH}_2\text{O-}$, $\text{-OCH}_2\text{-}$, -COO- , -OC=O- and $\text{-CH}_2\text{CHR}_1\text{-}$ (e.g. $\text{-CH}_2\text{CH}_2\text{-}$). Preferably, the X moiety nearest to the alpha atom is an NH or O atom, most preferably a NH group. The X moiety

alpha to the fused ring system is preferably a carbon based group such as CH_2 or CO , preferably CO . 1-amino-7-carbonylisoquinoline compounds optionally saturated between the 3 and 4 positions are novel and themselves
5 form a further aspect of the invention. 1-amino-7-substituted isoquinoline compounds optionally saturated between the 3 and 4 positions also form an aspect of the invention.

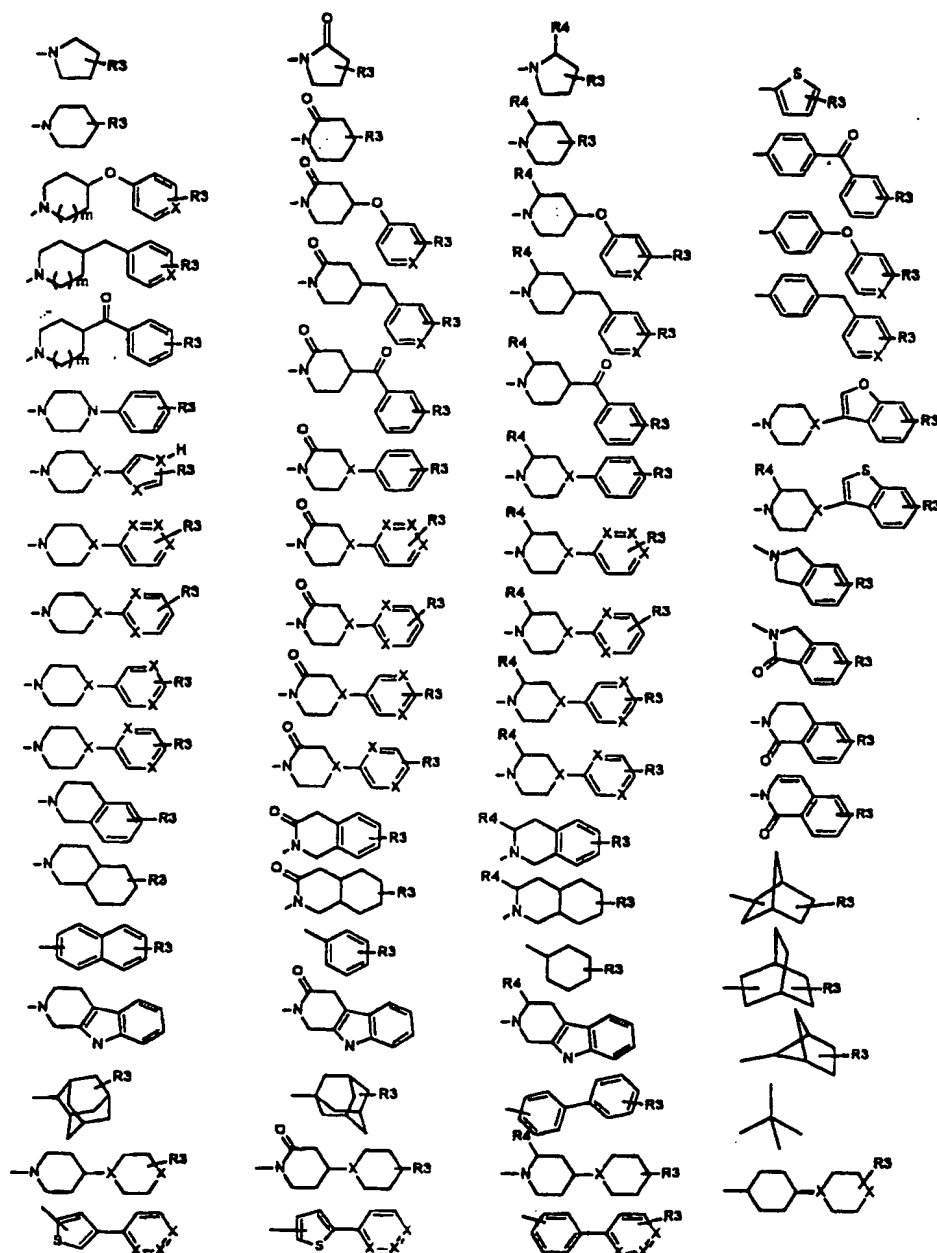
R_7 preferably represents an unsubstituted or R_1
10 substituted aryl or cyclohexyl group, preferably phenyl or naphthyl.

The linker group from the alpha atom to the lipophilic group is preferably CO , CH_2NH , $\text{CONR}_1(\text{CH}_2)_m$, $(\text{CH}_2)_m\text{N}(\text{R}_1)\text{CO}(\text{CH}_2)_m$, $(\text{CH}_2)_{m+2}$, $(\text{CH}_2)_m\text{CO}(\text{CH}_2)_m$, $(\text{CH}_2)_m\text{OC}=\text{O}$,
15 $(\text{CH}_2)_m\text{O}$ or $\text{CH}=\text{CH}(\text{CH}_2)_m$ (where each m is independently 0 or 1). The linker may be optionally branched, for example, to incorporate a polar functionality. In one embodiment Y and L taken together form a cyclic group and the alpha atom is therefore a carbon atom. The cyclic group can
20 be unsubstituted or substituted and can have a ring size of from 3 to 8 atoms. Preferably, the cyclic group is a cyclic amide, most preferably wherein the amide nitrogen of the cyclic amide group is bound to the lipophilic group.

The lipophilic group preferably comprises a cycloalkyl, azacycloalkyl, diazacycloalkyl, phenyl, naphthyl, adamantyl, decalinyl, tetrahydrodecalinyl, bicycloalkyl, mono- or diazabicycloalkyl, mono- or bicyclo heteroaromatic or a linear or branched alkyl,
30 alkylene, alkenyl or alkenylene group all optionally substituted by oxa, aza, thia or one or more groups R_1 , or a combination of at least two such groups linked by a spiro linkage or a single or double bond or by $\text{C}=\text{O}$, O , S , SO , SO_2 , CONR_1 , $\text{NR}_1\text{-CO-}$, NR_1 linkage. For example,
35 representative lipophilic groups include methylcyclohexyl, methylcyclohexylmethyl, methylphenylmethyl, phenylethyl, benzylpiperidinyl,

benzoylpiperidiny, bispiperidiny or phenylpiperaziny.

Most preferably, the lipophilic group is selected from



- 7 -

wherein R_3 is R_1 , aryl or cycloalkyl;

m represents 0 or 1;

R_4 represents hydrogen, $(CH_2)_wCOOH$, $(CH_2)_wCON(R_1)_2$,
(CH_2) $_wCON\alpha$ -AminoAcid;

5 w represents an integer from 0 to 4; and

X represents CH or N.

For example specific lipophilic groups include

10

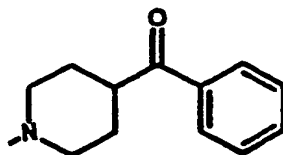
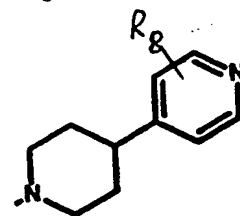
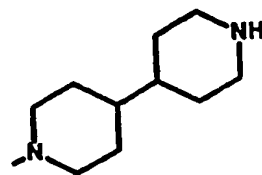
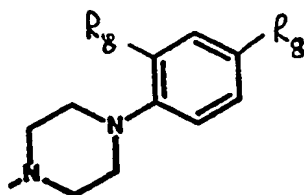
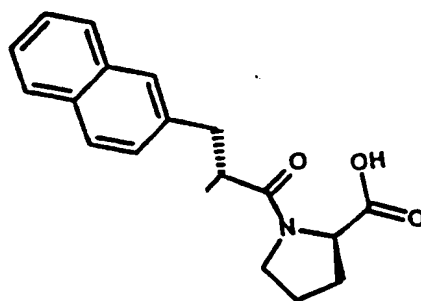
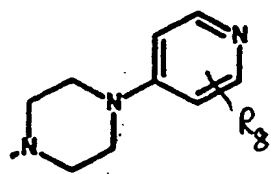
15

20

25

30

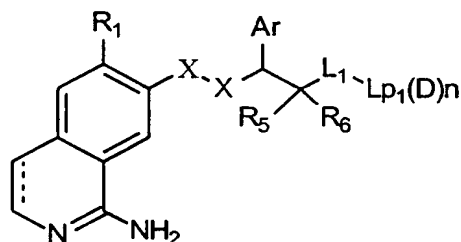
35



especially when R_8 represents H, OMe, F, NO_2 , OH or Cl.

The hydrogen bond donor group which may be attached to the lipophilic group preferably has a nitrogen or oxygen atom as the donor atom and conveniently is a hydroxyl group, a primary, secondary or tertiary amine, or a primary or secondary imine group (as part of an amidine or guanidine) or a saturated or unsaturated heterocyclic group containing a ring nitrogen, preferably a group containing 5 to 7 ring atoms. Where the donor atom is a ring nitrogen, the remote portion of the heterocyclic ring may be part of the lipophilic group.

Accordingly, preferred compounds of the invention are of formula



(wherein R_1 is as hereinbefore defined, R_1 preferably being hydrogen, hydroxy or amino);

R_5 and R_6 are hydrogen or taken together with the carbon atom to which they are attached represent a carbonyl group;

Ar is an unsubstituted or R_1 substituted aryl or cyclohexyl group, preferably phenyl or naphthyl;

X-X is -CONH-, -CH₂CH₂-, CH₂O-, -COO-, -CH₂NH-, -OCH₂- or -NHCH₂-;

L_1 is a valence bond or an organic linker group containing 1 to 4 backbone atoms selected from C, N and O;

L_{p1} is a cycloalkyl, azacycloalkyl, diazacycloalkyl, phenyl, naphthyl, adamantyl, decaliny, tetrahydrodecaliny, bicycloalkyl, mono- or diazabicycloalkyl, mono- or bicyclo heteroaromatic or a linear or branched alkyl, alkylene, alkenyl or

alkenylene group all optionally substituted by oxa, aza, thia or a group R_1 , or a combination of at least two such groups linked by a spiro linkage or a single or double bond or by $C=O$, O , S , SO , SO_2 , $CONR_1$, NR_1-CO- , NR_1

5 linkage. For example, representative lipophilic groups include a methyl-cyclohexyl, methylcyclohexylmethyl, methylphenylmethyl, phenylethyl, benzylpiperidinyl, benzoylpiperidinyl, bispiperidinyl or phenylpiperazinyl and those as hereinbefore described;

10 D is a hydrogen bond donor group;
and n is 0, 1 or 2).

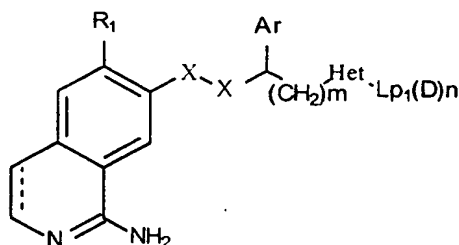
In one embodiment, L_1 comprises the backbone of an alpha amino acid, the lipophilic group being the side chain of the amino acid. The carboxyl part of the alpha
15 amino acid may be optionally coupled via an amide bond to an amino acid or to a primary or secondary cyclic or acyclic alkyl amine or diamine or via an ester bond to primary or secondary alcohols.

In a preferred embodiment, L_1 represents a valence
20 bond and the lipophilic group is bound directly to the carbonyl alpha to the alpha atom via a nitrogen atom which forms part of the lipophilic group. Suitable lipophilic groups in this case therefore include piperidinyl, pyrrolidinyl and piperazinyl. In a
25 preferred embodiment the piperidine or piperazinyl group is further substituted by a phenyl, benzyl, benzoyl, pyridyl, pyridyloxy, piperidinyl or phenoxy group, optionally substituted by one or more R_1 groups. Where the lipophilic group is pyridyl it is envisaged then N-
30 oxide pyridyl compounds will also be effective.

In a further embodiment, the lipophilic group has attached a group of the formula $-COOR_1$, $CON(R_1)_2$ or $-CON\alpha$ -aminoacid or ester derivative thereof.

In another embodiment the group binding the alpha
35 carbon atom to the lipophilic group comprises a heterocyclic group. Accordingly, preferred compounds of the invention also include

5



10 (wherein R_1 is as hereinbefore defined R_1 preferably being hydrogen, hydroxy or amino);

Ar is an unsubstituted or R_1 substituted aryl or cyclohexyl group, preferably phenyl or naphthyl;

X-X is -CONH-, -CH₂CH₂-, CH₂O-, -COO-, -CH₂NH-, -OCH₂- or -NHCH₂-;

15 m is 0, 1 or 2;

Het is a 5 or 6-membered heterocyclic group interrupted by 1, 2 or 3 heteroatoms selected from O, N and S optionally substituted by a group R_1 ;

20 Lp₁ is a cycloalkyl, azacycloalkyl, diazacycloalkyl, phenyl, naphthyl, adamantyl, decalinyl, tetrahydrodecalinyl, bicycloalkyl, mono- or diazabicycloalkyl, mono- or bicyclo heteroaromatic or a linear or branched alkyl, alkylene, alkenyl or alkenylene group all optionally substituted by a group R_1 , or a combination of at least two such groups linked by a spiro linkage or a single or double bond or by C=O, O, S, SO, SO₂, CONR₁, NR₁-CO-, NR₁ linkage; for example representative lipophilic groups include a cyclohexyl, phenyl, benzyl, benzyloxy or benzoyl;

30 D is a hydrogen bond donor group; and n is 0, 1 or 2).

35 Where Het is a five membered ring, the two ring atoms at which it is connected are preferably separated by one ring atom. Where Het is a six-membered ring, the two ring atoms at which it is connected are preferably separated by one or two ring atoms. Representative heterocyclic groups include thiazole, oxazole,

- 11 -

oxadiazole, triazole, thiadiazole or imidazole. Where the heterocyclic group is substituted by R_1 this is preferably a COOH , $\text{CON}(\text{R}_1)_2$ or COOR_1 connected to the heterocycle via a valence bond or alkylene chain.

5 In a further embodiment, the lipophilic group has attached a group of the formula $-\text{COOR}_1$ or $-\text{CON}\alpha\text{-aminoacid}$ or ester derivative thereof.

Especially preferred compounds according to the invention include 1-Aminoisoquinolin-7-oyl-D-phenylglyciny-D-2-naphthylalaninylproline, 1-10 Aminoisoquinolin-7-oyl-D-phenylglycine-4-(4'-pyridyl) piperidinamide, 1-Aminoisoquinolin-7-oyl-D-phenylglycine-4-methoxybenzylamide, 1-Aminoisoquinolin-7-oyl-D-phenylglycine-4-(4'-hydroxyphenyl)15 piperazinamide, 1-Aminoisoquinolin-7-oyl-D-phenylglycine-4-(2,4-difluorophenyl) piperazinamide, 1-Aminoisoquinolin-7-oyl-D-cyclohexylglycine-4-(4'-pyridyl) piperazinamide, 1-Aminoisoquinolin-7-oyl-D,L-1-naphthylglycine-4,4-bipiperidinamide, 2(R)-{[N-(1-20 Aminoisoquinoline)-7-oyl]amino}-2-phenylethyl-4-methylbenzamide and 1-Aminoisoquinolin-7-oyl-D-phenylglycine-4,4'-(1'-methyl)bipiperidinamide.

The use of aminoisoquinolines, particularly 1-amino-isoquinolines, more particularly 6 and/or 725 substituted isoquinolines, especially 7-substituted-1,6-diamino-isoquinolines, as serine protease inhibitors, especially factor Xa inhibitors, in particular in therapeutic or prophylactic treatments of humans or non-human animals, particularly mammals, is novel and forms30 a further aspect of the present invention.

The compounds of the invention may be prepared by conventional chemical synthetic routes, e.g. by amide bond formation to couple the fused bicyclic group to the lipophilic group. A preferred fused bicyclic group for35 coupling to the lipophilic group is 1-amino-7-carboxyisoquinoline which can be prepared from the readily available starting material 7-

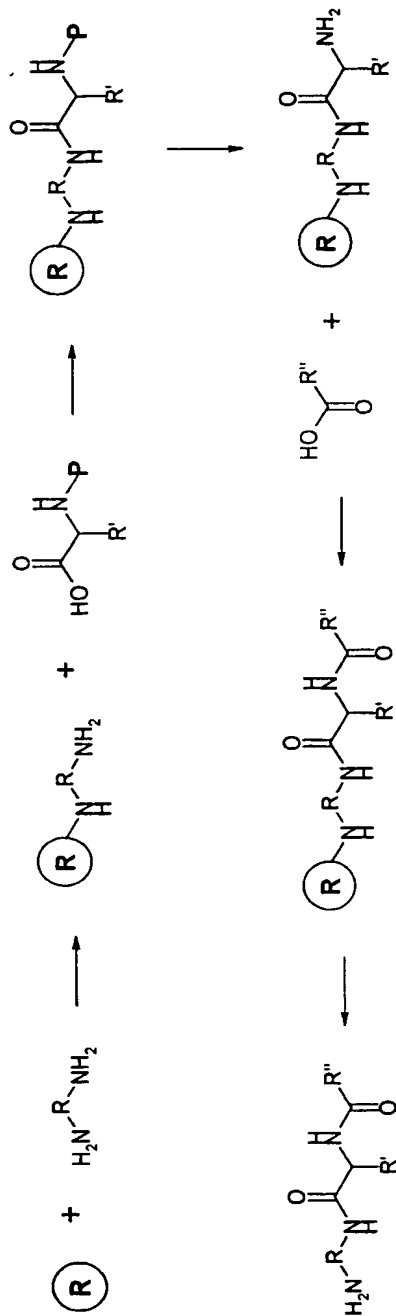
carboxyisoquinoline (F.T.Tyson, JACS, 1939, 61, 183). Amination of the 7-carboxyisoquinoline can be readily effected using ammonia as described in the Examples below.

5 Preparation of the lipophilic group is conveniently effected from for example LpNH_2 (where Lp is as hereinbefore defined). LpNH_2 may be attached to a resin to allow routine solid phase peptide synthesis to be carried out. The resin attached LpNH_2 moiety can then be
10 coupled by conventional techniques to a suitably protected amino acid, whose side chain will form the R_1 group, via for example a free amino group in the Lp group. Deprotection can then be effected before
15 coupling to 1-amino-7-carboxyisoquinoline and isolation from the resin. This method is illustrated in Scheme 1 below.

 Alternatively, the synthesis can be initiated from an LpCOOH derivative and the carboxyl functionality can be coupled to the resin. Conventional peptide synthesis
20 as illustrated in Scheme 2 can then be carried out.

 Further peptide based syntheses are illustrated in Schemes 3 to 5.

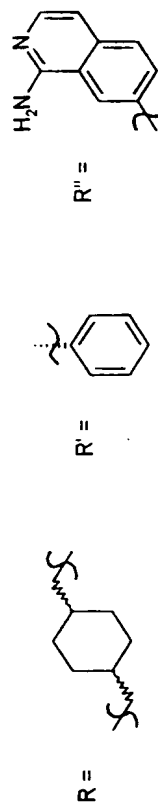
Scheme 1



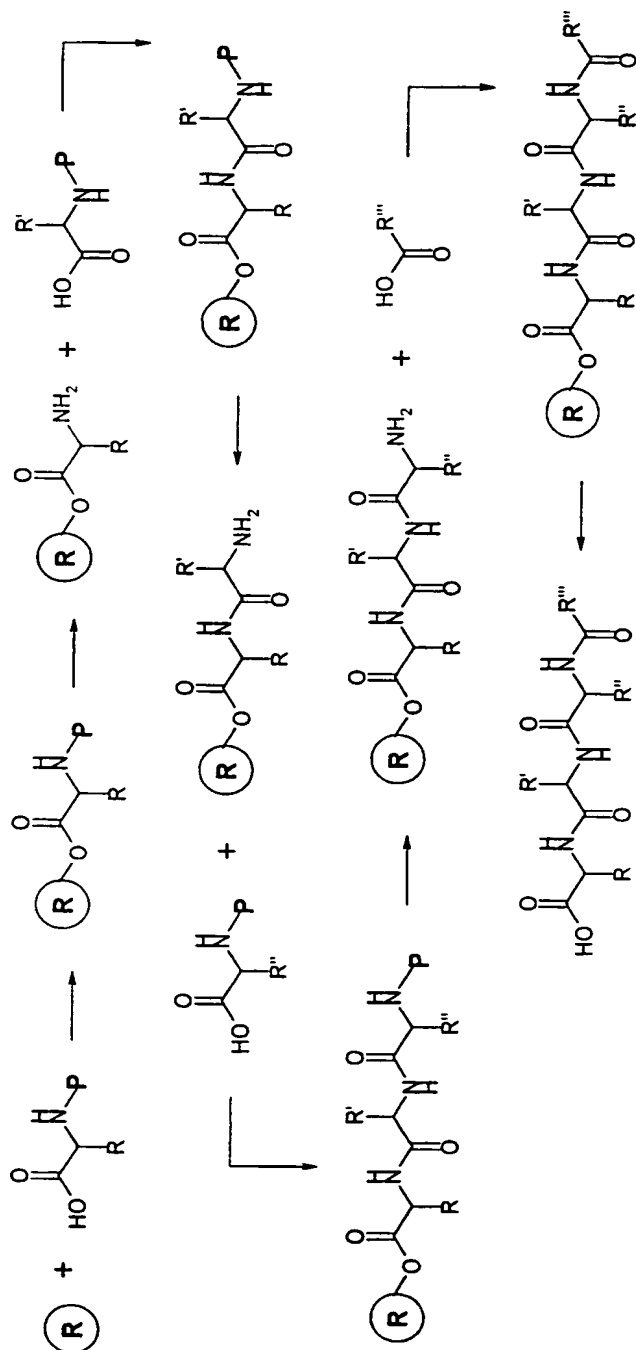
R - denotes resin

P - denotes protecting group

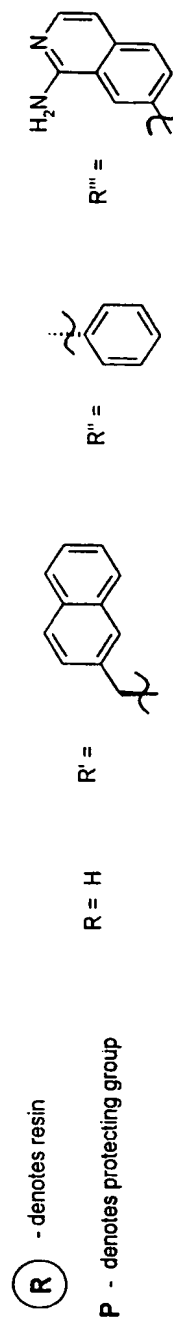
In the example



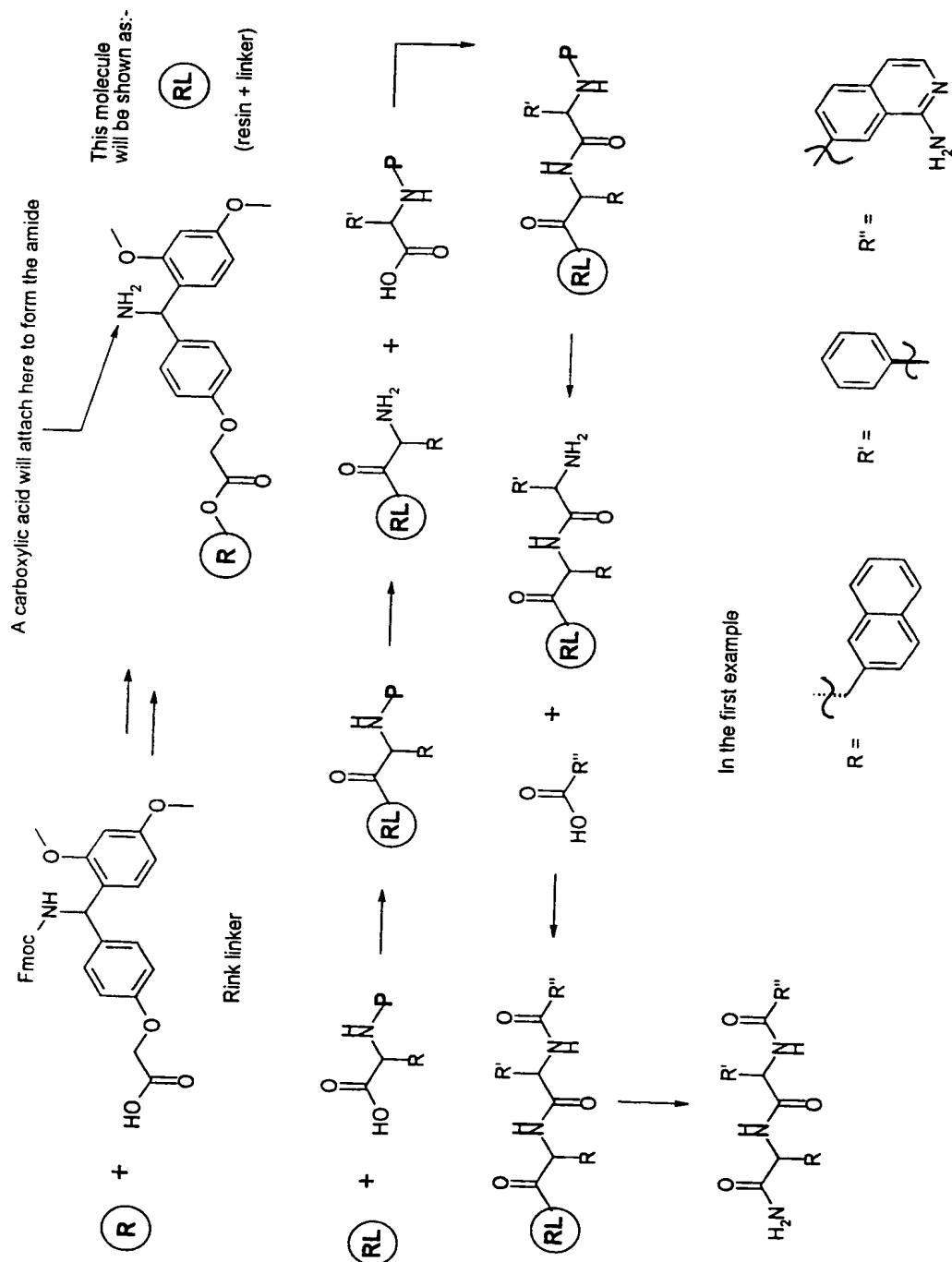
Scheme 2

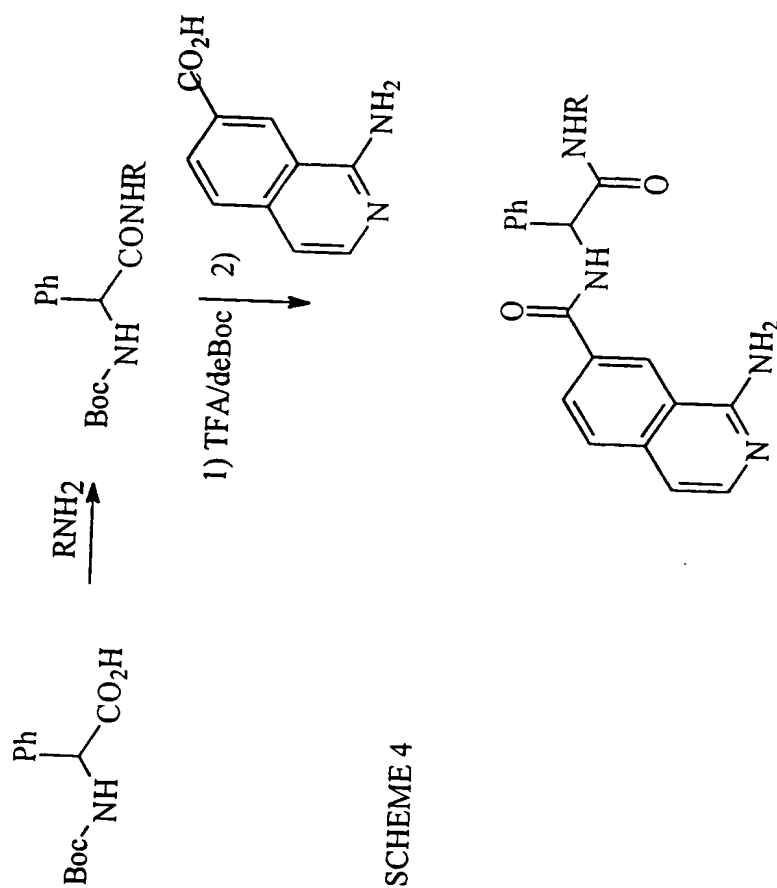


In the first example

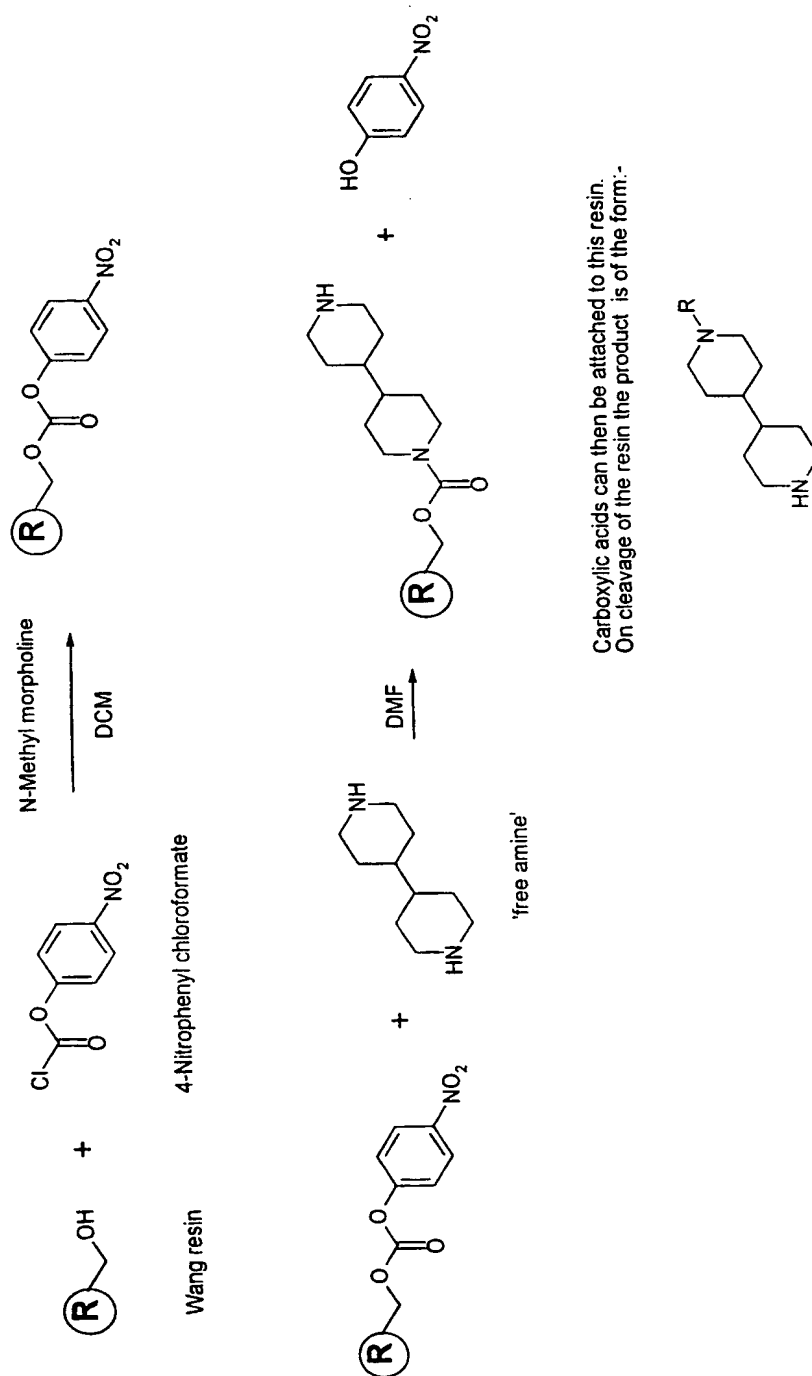


Scheme 3





Scheme 5



In all the syntheses illustrated in Schemes 1 to 5 the coupling reactions can of course be effected using an activated form of the carbonyl compound e.g. an acid chloride or active ester.

5 Where Y and L taken together form a cyclic amide group this can be conveniently introduced by reacting an Lp group carrying a secondary amine with an active side chain with a suitable amino acid. Cyclisation can be
10 base induced via nucleophilic attack of the alpha atom on a leaving group on the active side chain.

If necessary the amide linkage from isoquinoline to alpha atom can be reduced using an appropriate reducing agent employing the necessary protection.

15 Alternatively, the compounds of the invention where X-X is C-N or C-O can be prepared from compounds of formula $H_2NCH(R_7)R''$ or $HOCH(R_7)R''$ respectively, by condensation with an appropriately substituted isoquinoline, e.g. 1-amino-7-bromomethyl-isoquinoline, in the presence of a base, where R'' is L-Lp(D)_n or a
20 functionality which can be converted to such by methods herein described.

Compounds of the invention where X-X is C-N can also be prepared from compounds of the formula $H_2NCH(R^7)R''$ by condensation with an appropriately
25 substituted isoquinoline aldehyde or ketone, e.g. 1-amino-isoquinoline-7-carboxaldehyde, in the presence of a reducing agent such as sodium borohydride or sodium triacetoxyborohydride.

30 Alternatively, the compounds of the invention be prepared from compounds of formula $NH_2CH(R_7)COOH$ which can be conveniently reduced to an alcohol by reaction with isobutylchloroformate and reduction with sodium borohydride using suitable protection if necessary.

35 Such an alcohol can be reacted using suitable protection, to introduce the Lp group by reactions such as:

alkylation with an alkyl halide in the presence of

a base;

reaction with diethyl
azodicarboxylate/triphenylphosphine and a hydroxylated
aryl compound;

5 by reaction with an activated carboxylic acid (e.g.
an acid chloride) or with a carboxylic acid and
diethylazodicarboxylate/triphenylphosphine;

by reaction with an isocyanate; and

10 by treatment with methanesulphonyl chloride or
trifluoromethanesulphonic anhydride and reaction with an
amine, or with a thiol optionally followed by oxidation,
e.g. with potassium metaperiodate or hydrogen peroxide.

In this way compounds with linkages of $-\text{CH}_2-\text{O}-$,
 $-\text{CH}_2-\text{O}-\text{CO}-$, $-\text{CH}_2-\text{O}-\text{CO}-\text{NR}_1-$, $-\text{CH}_2-\text{NR}_1-$, $-\text{CH}_2-\text{S}-$, $-\text{CH}_2-\text{SO}-$
15 and $-\text{CH}_2-\text{SO}_2-$ between the alpha carbon and the lipophilic
group may be produced. These compounds can then be
coupled with 1-amino-7-isoquinoline carboxylic acid TFA
salt.

Alternatively the alcohol can be oxidized to form a
20 corresponding aldehyde (e.g. by oxidation with manganese
dioxide or DMSO/oxalyl chloride or DMSO/ SO_3 or Dess-
Martin reagent) which may be reacted to introduce the Lp
group by reactions such as:

reaction with Wittig reagents or Horner-Emmons
25 reagents, optionally followed by reduction of the
resulting carbon:carbon double bond using H_2/Pd -carbon;

reaction with an organometallic, eg a Grignard
reagent, optionally followed by reaction on the
resulting hydroxyl group, such as oxidation (eg with
30 MnO_2 , DMSO/oxalyl chloride or Dess-Martin reagent),
alkylation (eg with an alkyl halide in the presence of a
base in a solvent such as DMF), arylation (eg with
diethyl-azodicarboxylate/triphenyl phosphine and a
hydroxyaryl compound), ester formation (eg with an acid
35 chloride or with a carboxylic acid and diethylazido
dicarboxylate/triphenyl phosphine), or carbamate
formation (eg with an isocyanate);

- 20 -

by reaction with an amine followed by reduction,
e.g. with sodium cyanoborohydride;
by reaction with a hydrazine; or
by reaction with a carbazide.

5 In this way compounds with linkages of $-\text{CH}=\text{CR}_1-$,
 $-\text{CH}_2-\text{CHR}_1-$, $-\text{CHOH}-$, $-\text{CHR}_1-\text{O}-$, $-\text{CHR}_1-\text{O}-\text{CO}-$, $-\text{CHR}_1-\text{O}-\text{CO}-\text{NR}_1-$,
 $-\text{CO}-$, $-\text{CH}_2-\text{NR}_1-$, $-\text{CH}=\text{N}-\text{NR}_1-$ and $-\text{CH}=\text{N}-\text{NR}_1-\text{CO}-\text{NR}_1-$ between
the alpha carbon and the lipophilic group may be
produced. Again the resulting compounds could then be
10 coupled to the isoquinoline derivative.

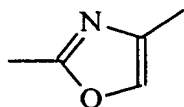
The transformation of alcohol to amine referred to
above may be used to produce an amine reagent for
lipophilic group introduction, e.g. a compound NH_2-
 $\text{CH}(\text{R}_7)-\text{CH}_2-\text{NR}_1\text{H}$.

15 Such an amine reagent may be reacted to introduce
the lipophilic group, e.g. by acylation with an acid
halide or activated ester, by reaction with isocyanate,
by reaction with an isothiocyanate, or by reaction with
a sulphonyl chloride. In this way compounds with
20 linkages of $-\text{CH}_2\text{NR}_1-\text{CO}-$, $-\text{CH}_2-\text{NR}_1-\text{CO}-\text{NR}_1-$, $-\text{CH}_2\text{NR}_1-\text{CS}-\text{NR}_1-$
and $-\text{CH}_2\text{NR}_1-\text{SO}_2-$ between the alpha carbon and the
lipophilic groups may be produced.

The transformation of acid to amide referred to
above may be used to produce an amide reagent for
25 introduction of the Lp group, e.g. a compound $\text{NH}_2-\text{CH}(\text{R}_1)-$
 CONH_2 .

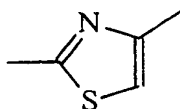
Such amides may be reacted to introduce lipophilic
groups, e.g. by reaction with a haloketone (e.g.
phenacyl bromide). This provides a linkage

30

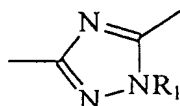


from alpha carbon to lipophilic group.

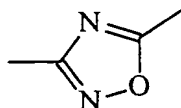
35 Analogously the amide may be transformed to a
thioamide by reaction with Lawesson's reagent and then
reacted with a haloketone to form a linkage



5 The amide reagent may likewise be transformed to a nitrile reagent by dehydration, e.g. with trifluoroacetic anhydride. The nitrile reagent may be reacted with hydrazine then with acyl halide and then cyclized, (e.g. with trifluoroacetic anhydride) to
10 produce a linkage

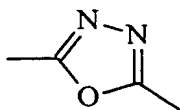


15 Alternatively it may be treated with hydroxylamine then reacted with acyl halide and cyclized (e.g. with trifluoroacetic anhydride) to produce a linkage



20 The hydrazide produced by reaction of a carboxylic acid reagent with hydrazine discussed above may likewise be used as a reagent for lipophilic group introduction,
25 e.g. as a compound of formula $\text{NH}_2\text{-CH(R}_1\text{)-CO-NR}_1\text{-N(R}_1\text{)}_2$.

 Thus the hydrazide reagent can be reacted with an acyl halide and cyclized, e.g. with trifluoroacetic anhydride to yield a linkage



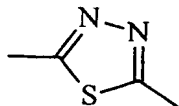
30 or reacted with an acyl halide or an isocyanate to yield linkages $\text{-CO-NR}_1\text{-NR}_1\text{-CO-}$ and $\text{-CO-NR}_1\text{-NR}_1\text{-CO-NR}_1\text{-}$
35 respectively.

 Alternatively the hydrazide may be transformed by reaction with Lawesson's reagent and then reacted with

- 22 -

an acyl halide and cyclized (e.g. with trifluoroacetic anhydride) to produce the linkage

5



10 An alternative route to these compounds is to carry out any of the above chemical reactions to incorporate the lipophilic group (and optional H bond donor) into a protected intermediate such as a compound of formula (IV).

15

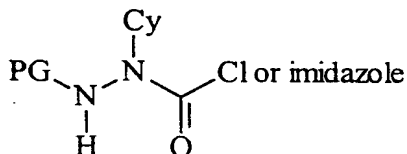


PG = Protecting group

20 The protecting group may then be removed before coupling of the 1-amino-7-carboxyisoquinoline (optionally protected).

25 A starting reagent for lipophilic group introduction where the alpha atom is nitrogen may be produced for example by reaction of a beta protected hydrazine (such protection to be chosen as to be compatible with the subsequent reagents to be employed) with phosgene, diphosgene, triphosgene or N,N'carbonyl diimidazole to give a reactive compound of the type:

30



35

PG = Protecting group

- 23 -

This intermediate may be used as has been described above for the carboxylic starting reagents where the alpha atom is carbon.

Removal of the protecting group by standard methods and coupling with an activated 1-amino-7-carboxyisoquinoline will give compounds of the type



(where R_7 , L, Lp and D are as defined above and IQ is a 1-aminoisoquinoline linked in the 7-position).

Thus viewed from a further aspect the invention provides a process for the preparation of a compound according to the invention which process comprises coupling a carbonyl attached group to 1-amino-7-carboxyisoquinoline.

The compounds of the invention may be administered by any convenient route, e.g. into the gastrointestinal tract (e.g. rectally or orally), the nose, lungs, musculature or vasculature or transdermally. The compounds may be administered in any convenient administrative form, e.g. tablets, powders, capsules, solutions, dispersions, suspensions, syrups, sprays, suppositories, gels, emulsions, patches etc. Such compositions may contain components conventional in pharmaceutical preparations, e.g. diluents, carriers, pH modifiers, sweeteners, bulking agents, and further active agents. Preferably the compositions will be sterile when in a solution or suspension form suitable for injection or infusion. Such compositions form a further aspect of the invention.

Viewed from this aspect the invention provides a pharmaceutical composition comprising a serine protease inhibitor according to the invention together with at least one pharmaceutically acceptable carrier or excipient.

Viewed from a further aspect the invention provides the use of a serine protease inhibitor according to the

- 24 -

invention for the manufacture of a medicament for use in a method of treatment of the human or non-human animal body (e.g. a mammalian, avian or reptilian body) to combat a condition responsive to a serine protease inhibitor (e.g. a condition such as a thrombotic disorder responsive to a factor Xa inhibitor), said method comprising administering to said body an effective amount of a serine protease inhibitor according to the invention.

The dosage of the inhibitor compound of the invention will depend upon the nature and severity of the condition being treated, the administration route and the size and species of the patient. However in general, quantities of from 0.01 to 100 $\mu\text{mol/kg}$ bodyweight will be administered.

All publications referred to herein are hereby incorporated by reference.

The invention will now be described further with reference to the following non-limiting Examples.

Experimental

Abbreviations used follow IUPAC-IUB nomenclature. Additional abbreviations are Hplc, high-performance liquid chromatography; DMF, dimethylformamide; DCM, dichloromethane; HAOt, 1-hydroxy-7-azabenzotriazole; HATU, [O-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate]; Fmoc, 9-Fluorenylmethoxycarbonyl; HOBT, 1-hydroxybenzotriazole; TBTU, 2-(1H-(benzotriazol-1-yl)-1,1,3,3-tetramethyluroniumtetrafluoroborate; DIPEA, diisopropylethylamine; Boc, tertiary butyloxycarbonyl; DIPCI, diisopropylcarbodiimide; DBU, 1,8-diazabicyclo[5.4.0]undec-7-ene; TEA, triethylamine; EDC, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride; Rink linker, p-[(R,S)- α -[1-(9H-Fluoren-9-yl)methoxyformamido]-2,4-dimethoxybenzyl]phenyl acetic

- 25 -

acid; TFA, trifluoroacetic acid; MALDI-TOF, Matrix assisted laser desorption ionisation - time of flight mass spectrometry; and RT, retention time. Unless otherwise indicated amino acid derivatives, resins and coupling reagents were obtained from Novabiochem (Nottingham, UK) and other solvents and reagents from Rathburn (Walkerburn, UK) or Aldrich (Gillingham, UK) and were used without further purification. All solution concentrations are expressed as %Vol./%Vol. unless otherwise stated.

Purification: Purification was by gradient reverse phase Hplc on a Waters Deltaprep 4000 at a flow rate of 50 ml/min. using a Deltapak C18 radial compression column (40 mm x 210 mm, 10-15 mm particle size). Eluant A consisted of aqTFA (0.1%) and eluant B 90% MeCN in aqTFA(0.1%) with gradient elution (Gradient 1, 0 min. 20%B then 20% to 100% over 36 min., Gradient 2, 0 min. 5%B for 1 min. then 5%B to 20%B over 4 min., then 20% to 60% over 32 min. or Gradient 3, 0 min. 20%B then 20% to 100% over 15 min.). Fractions were analysed by analytical Hplc and MALDI-TOF or electrospray LCMS before pooling those with >95% purity for lyophilisation.

Analysis: Analytical Hplc was on a Shimadzu LC6 gradient system equipped with an autosampler, a variable wavelength detector at flow rates of 0.4 ml/ min. Eluents A and B as for preparative Hplc. Columns used were Techogell15 C18 (2x150mm) (Hplc Technology), Magellan C8 column (2.1x150 mm, 5mm particle size) (Phenomenex). Purified products were further analysed by MALDI-TOF and nmr.

35 **Synthesis of inhibitors**

Method 1: Using a solid phase strategy on a Protein

- 26 -

Technologies, Symphony Multiple Peptide Synthesiser by attachment of bis amino compounds to Peg-2-chlorotrityl chloride resin: 2-Chlorotrityl chloride resin was typically treated with greater than 2 fold excess of the di-amine in dry DCM. The resin was further modified by the attachment of acids. Activation of Fmoc protected amino acid (2-5eq) was by TBTU/ DIPEA, all couplings (minimum 120 min.) were carried out in DMF. Deprotection of the Fmoc group was achieved with 20% piperidine in DMF. Other acid substituents were added as the HOBt or HOAt esters either by activation with HBTU/HATU or DIPCI with or without Boc protection of amino groups. Cleavage of the products from the resin was by treatment (30 min., ambient) with 10% triethylsilane in TFA, filtration, evaporation and trituration with diethylether.

Synthesis using the Symphony Multiple Peptide Synthesiser.

The Symphony Multiple Peptide Synthesiser is charged with DMF, DCM, TBTU in DMF (450 mM), DIPEA in DMF (900 mM), 20% piperidine in DMF. Resins are held in plastic reaction vessels that allow the introduction of reagents and solvents and nitrogen for agitation or air drying.

A typical synthesis cycle on the Symphony is as follows:-

The reaction vessel containing the resin (0.1 mmol) is charged with the Fmoc protected amino acid (0.5 mmol) and then this is dissolved in DMF (2.5ml), treated with TBTU (0.56 mmol, 1.25ml) and DIPEA (1.1 mmol, 1.25ml) and agitated with nitrogen for 2 hours (agitation times may vary). After coupling, the resin is washed with DMF (6x 5ml) then deprotected with 20% piperidine in DMF (2x 5ml for 1 min. each, then 1x 5ml for 8 min.). The resin

- 27 -

is then washed with DMF (6x 5ml).

Example 1**1-Aminoisoquinolin-7-oyl-D-phenylglycine-4-aminomethylcyclohexyl methylamide**

Bis-1,4-aminomethylcyclohexane(2ml) was added to 2 chlorotrityl chloride resin (1.2 mmol/g, 0.73g) pre swollen in dry DCM (4ml). After 2h the resin was washed with DCM (6x5ml), DMF (6x5ml) and DCM (6x5ml). The resin was then air dried to allow aliquots to be taken.

The bis-1,4-aminomethylcyclohexane 2-chlorotrityl resin (0.1 mmol) was treated with Fmoc-D-Phenylglycine (0.5 mmol, 187mg), DMF(2.5ml), TBTU in DMF(1.25ml of a 450mM solution) and DIPEA in DMF (1.25ml of a 900 mM solution). The mixture was agitated with nitrogen for 2 hours. Deprotection and washing as above.

A solution of 1-aminoisoquinoline-7-carboxylic acid trifluoroacetic acid salt (150mg 0.5mmole) (Example 88) in dry dimethylformamide (DMF) was treated successively with HOAt (102mg 0.75mmole) and EDC (115mg 0.6mmole) and stirred at room temperature for 10min. The mixture was transferred to the reaction vessel on the Symphony and agitated for 10 hours with nitrogen. The resin was washed with DMF (6x5ml), DCM (6x5ml) and air dried. The product was cleaved from the resin with 10% triethylsilane in TFA (10ml) for 30 minutes, the resin filtered off and the TFA solution evaporated to dryness and triturated with diethyl ether to give the crude product. The crude product was dissolved in water (10ml), filtered and purified by preparative reverse phase Hplc.

¹H nmr (CD₃CN) Mixture of cyclohexyl cis and trans isomers. 8.65 (1H,s); 8.10 (1H,d); 7.75 (1H,d); 7.35

- 28 -

(1H,d); 7.30 (2H,m); 7.15 (3H, m); 7.00 (1H,d); 5.40 (1H, s); 3.25-2.55 (4H, m); 1.7-0.9 (7H, m); 0.7-0.5 (3H, m) MS TOF 446 (M+1⁺). Hplc (Magellan C8, Gradient 3, water/acetonitrile/TFA) rt 8.23 min.

5

Method 2: By solid phase strategy on a Protein Technologies, Symphony Multiple Peptide Synthesiser using Fmoc amino acids attached to Peg-2-chlorotrityl chloride resin: Typically the 2-chlorotrityl chloride resin was treated with a 2 fold excess of the Fmoc amino acid in a 1:1 mixture of DMF and dry DCM and DIPEA (2eq.). The resin was washed with DMF/DCM and deprotected with 20% piperidine in DMF before further modification. The resin was further modified by the attachment of acids. Activation of Fmoc protected amino acids (2-5eq) was by activation with TBTU/ DIPEA, all couplings (minimum 120 min.) were carried out in DMF. Deprotection of the Fmoc group was achieved with 20% piperidine in DMF. Other acid substituents were added as the HOBt or HOAt esters either by activation with HBTU/HATU or DIPCI with or without Boc protection of amino groups. Cleavage of the products from the resin was by treatment (30 min., ambient) with 10% triethylsilane in TFA, filtration, evaporation and trituration with ether.

25

Example 2

1-Aminoisoquinolin-7-oyl-D-phenylglyciny-D-2-naphthylalaninyglycine

30

Fmoc Glycine (0.2mmol, 59 mg.) in DMF (2ml) was added to 2 chlorotrityl chloride resin (1.0 mmol/g, 0.1g) pre swollen in dry DCM (2ml), then DIPEA (0.2 mmol,). After 2h the resin was washed with DCM (6x5ml), DMF (6x5ml) and DCM (6x5ml). The resin was then air dried to allow aliquots to be taken for further modification.

35

- 29 -

On the Symphony Fmoc-Glycyl-2-chlorotrityl resin (0.1 mmol) was deprotected with 20% piperidine in DMF and washed with DMF (6x 5ml) then treated with Fmoc-D-2-naphthylalanine (0.5 mmol 220 mg), DMF (2.5ml), TBTU in DMF (1.25ml of a 450mM solution) and DIPEA in DMF (1.25ml of a 900 mM solution). The mixture was agitated with nitrogen for 2 hours. Deprotection and washing as above. The resin was then treated Fmoc-D-Phenylglycine (0.5 mmol 187 mg), DMF (2.5ml), TBTU in DMF (1.25ml of a 450mM solution) and DIPEA in DMF (1.25ml of a 900 mM solution). The mixture was agitated with nitrogen for 2 hours. Deprotection and washing as above.

A solution of 1-aminoisoquinoline-7-carboxylic acid trifluoroacetic acid salt (150mg 0.5mmole) (Example 88) in dry dimethylformamide (DMF) was treated successively with HOAt (102mg 0.75mmole) and EDC (115mg 0.6mmole) and stirred at room temperature for 10min. The mixture was transferred to the reaction vessel on the Symphony and agitated for 10 hours with nitrogen. The resin was then washed with DMF (6x5ml), DCM (6x5ml) and air dried. The product was cleaved from the resin with 10% triethylsilane in TFA (10ml) for 30 minutes, the resin filtered off, the TFA solution evaporated to dryness and triturated with diethyl ether to give the crude product. The crude product was then dissolved in water (10ml), filtered and purified by preparative reverse phase Hplc.

¹H nmr (DMSO) 8.80 (1H,s); 8.20 (1H,d); 7.85 (1H,d); 7.55 (6H,m); 7.35 (1H, d); 7.20 (7H, m); 5.80 (1H, s); 4.70 (1H,m); 3.85 (2H,m, obscured by solvent); 3.15 (2H,m). MS TOF 576 (M+1+). Hplc (Magellan C8, Gradient 3, water/acetonitrile/TFA) rt 10.98 min.

Other compounds made by the above method:-

- 30 -

Example 3**1-Aminoisoquinolin-7-oyl-D-phenylglyciny-D-2-naphthylalaninyl-D-proline**

5 1H nmr (DMSO) 8.35 (1H,s); 7.90 (1H,d); 7.65 (1H,d);
7.45 (5H,m); 7.35 (1H, d); 7.20 (1H,d); 7.05 (7H, m);
5.45 (1H, s); 4.75 (1H,m); 4.10 (1H,m); 3.45 (2H,m,
obscured by solvent); 3.00 (2H,m) 2.00 (1H,m); 1.70
10 (2H,m); 1.45 (1H,m). MS TOF 576 (M+1+). Hplc (Magellan
C8, Gradient 3, water/acetonitrile/TFA) rt 11.19 min.

Method 3: By solid phase strategy on a Protein
Technologies, Symphony Multiple Peptide Synthesiser
using Fmoc amino acids attached to TentaGel S-resin
15 (Rapp Polymere) via the Rink amide linker: Typically the
TentaGel resin was treated with a 5 fold excess of the
Rink linker, TBTU (1eq.), DIPEA (2eq.) in DMF for a
minimum of 120mins. The resin was washed with DMF and
deprotected with 20% piperidine in DMF before further
20 modification. The resin was further modified by the
attachment of acids. Activation of Fmoc protected
amino acid (2-5eq) was by TBTU/DIPEA, all couplings
(minimum 120 min.) were carried out in DMF.
Deprotection of the Fmoc group was achieved with 20%
25 piperidine in DMF. Other acid substituents were added
as the HOBT or HOAt esters either by activation with
HBTU/HATU or DIPCI with or without Boc protection of
amino groups. Cleavage of the products from the resin
was by treatment (30 min., ambient) with 10%
30 triethylsilane in TFA, filtration, evaporation and
trituration with ether.

Example 4**1-Aminoisoquinolin-7-oyl-D-phenylglyciny-D-2-naphthylalanine amide**
35

On the Symphony TentaGel S -NH₂ resin (0.1 mmol, 400 mg,

- 31 -

0.24 mmol./g) was treated with Rink linker (0.5 mmol, 270mg), DMF(2.5ml), TBTU in DMF(1.25ml of a 450mM solution) and DIPEA in DMF (1.25ml of a 900 mM solution). The mixture was agitated with nitrogen for 2 hours. Deprotection and washing as above.

On the Symphony Rink-TentaGel resin (0.1 mmol) was then treated with Fmoc-D-naphthylalanine (0.5 mmol, 194mg), DMF(2.5ml), TBTU in DMF(1.25ml of a 450mM solution) and DIPEA in DMF (1.25ml of a 900 mM solution). The mixture was agitated with nitrogen for 2 hours. Deprotection and washing as above.

The resin (0.1 mmol) was then treated with Fmoc-D-Phenylglycine (0.5 mmol, 187mg), DMF(2.5ml), TBTU in DMF(1.25ml of a 450mM solution) and DIPEA in DMF (1.25ml of a 900 mM solution). The mixture was agitated with nitrogen for 2 hours. Deprotection and washing as above.

A sample of 1-aminoisoquinoline-7-carboxylic acid trifluoroacetic acid salt (150mg 0.5mmole) (Example 88) in dry dimethylformamide (DMF) was treated successively with HOAt (102mg 0.75mmole) and EDC (115mg 0.6mmole) and stirred at room temperature for 10min. The mixture was then transferred to the reaction vessel on the Symphony and agitated for 10 hours with nitrogen. The resin was then washed with DMF (6x5ml), DCM (6x5ml) and air dried. The product was cleaved from the resin with 10% triethylsilane in TFA (10ml) for 30 minutes, the resin filtered off, the TFA solution evaporated to dryness and triturated with diethyl ether to give the crude product. The crude product was then dissolved in water (10ml), filtered and purified by preparative reverse phase Hplc.

¹H nmr (CD₃CN) 8.45 (1H,s); 8.05 (1H,d); 7.85 (1H,d); 7.55 (5H,m); 7.45 (1H, d); 7.25 (1H,d); 7.10 (7H, broad

- 32 -

m); 5.45 (1H, s); 4.65 (1H,m); 3.15 (2H,m). MS TOF 518 (M+1⁺). Hplc (Magellan C8, Gradient 3, water/acetonitrile/TFA) rt 10.67 min.

5 **Method 4:** By solution phase strategy: Typically an activated Boc-amino acid was treated with an amine (primary or secondary) or alcohol (1eq.). Activation of Boc protected amino acid was by HATU or TBTU/DIPEA(1:2), all couplings (minimum 120 min.) were
10 carried out in DMF. After an aqueous work up the deprotection of the Boc group was achieved with TFA. Other acid substituents were added as the HOBT or HOAt esters either by activation with HBTU/HATU, EDC or DIPCI with or without Boc protection of amino groups. The
15 final products were purified by preparative reverse phase Hplc.

Example 5

20 **1-Aminoisoquinolin-7-oyl-D-phenylglycine-4-methylbenzylamide**

Boc D-phenylglycine (251 mg, 1 mmol.) was dissolved in DMF(3ml) with HATU (380 mg., 1 mmol.) and DIPEA(350µl., 2 mmol.). To this mixture was added 4-methylbenzyl-
25 amine(121mg., 1 mmol.) and DIPEA (170µl., 1 mmol.). The mixture was stirred overnight. The mixture was then taken up into ethylacetate and washed with water, sodium carbonate solution, water, 10% hydrochloric acid solution and water. The ethylacetate was evaporated
30 without drying and treated immediately with TFA for 30 min. The TFA was then evaporated to dryness and the product triturated with diethylether. TEA(1ml) was added and evaporated to dryness. A solution of 1-aminoisoquinoline-7-carboxylic acid trifluoroacetic acid
35 salt (300mg 1mmole) (Example 88) in dry dimethylformamide (DMF) was treated successively with HOAt (204mg 1.5mmole) and EDC (230mg 1.2mmole) and

- 33 -

stirred at room temperature for 10min. The mixture was then added to the D-phenylglycine-4-methylbenzylamide and stirred overnight. The crude product was dissolved in water/acetonitrile (20ml), filtered and purified by preparative Hplc to yield pure product.

1H nmr (CD₃CN) 8.86 (1H, s); 8.32 (1H, d); 7.96 (1H, d); 7.60 (1H, d); 7.46 (5H, m); 7.24 (1H, d); 7.06 (4H, m); 5.64 (1H, s); 4.31 (2H, m); 2.29 (3H, m). MS TOF 426 (M+1⁺). Hplc (Magellan C8, Gradient 3, water/acetonitrile/TFA) rt 11.00 min.

Other compounds made by the above method:-

Example 6

1-Aminoisoquinolin-7-oyl-D-phenylalanine-4-methylbenzylamide

1H nmr (CD₃CN) 8.65 (1H, s); 8.05 (1H, d); 7.80 (1H, d); 7.45 (1H, d); 7.10 (6H, m); 6.95 (4H, m); 4.70 (1H, m); 4.15 (2H, m); 2.95 (2H, m); 2.20 (3H, m). MS TOF 440 (M+1⁺). Hplc (Magellan C8, Gradient 3, water/acetonitrile/TFA) rt 11.40 min.

Example 7

1-Aminoisoquinoline-7-oyl-D-phenylglycine-4-benzoyl piperidinamide

1H nmr (CD₃CN) mixture of rotomers 8.85 (1H, d); 8.25 (1H, m); 8.00 (1H, s); 7.85 (2H, m); 7.58 (2H, m); 7.40 (7H, m); 7.20 (1H, d); 6.03 (1H, s); 4.50 (1H, m); 3.15 (1H, m); 2.80 (3H, m); 1.60 (2H, m); 1.42 (1H, m); 0.45 (1H, m). MS TOF 493 (M+1⁺). Hplc (Magellan C8, Gradient 3, water/acetonitrile/TFA) rt 11.48 min.

Example 8**1-Aminoisoquinolin-7-oyl-D-phenylglycine-4-(4'-pyridyl) piperazinamide**

5 1H nmr (CD₃CN) 8.70 (1H,s); 8.15 (1H,d); 7.90 (2H, d);
7.85 (1H, d); 7.45 (1H, d); 7.35 (5H, m); 7.10 (1H,d);
6.80 (2H,d); 6.00 (1H, s); 3.70-3.10 (broad 8H,m). MS
TOF 467 (M+1⁺). Hplc (Magellan C8, Gradient 1,
water/acetonitrile/TFA) rt 9.00 min.

10

Example 9**1-Aminoisoquinolin-7-oyl-D-phenylglycine-4-(4'-aminophenyl) piperazinamide**

15 1H nmr (CD₃CN) 8.80 (1H,s); 8.25 (1H,d); 7.95 (1H, d);
7.55 (1H, d); 7.35 (5H, m); 7.20 (3H, d); 7.00 (2H,d);
6.10 (1H, s); 3.70-2.60 (several broad 8H,m). MS TOF
481 (M+1⁺). Hplc (Magellan C8, Gradient 3,
water/acetonitrile/TFA) rt 9.91 min.

20

Example 10**1-Aminoisoquinolin-7-oyl-D-phenylglycine-4-phenyl piperazinamide**

25 1H nmr (CD₃CN) 8.70 (1H,d); 8.25 (1H,d); 7.90 (1H, d);
7.60 (3H, d); 7.40 (3H, m); 7.25 (2H, m); 7.15 (1H, d);
6.90 (3H,m); 6.15 (1H, s); 3.85 (1H, m); 3.70 (2H,m);
3.55 (1H,m); 3.15 (3H,m); 2.65 (1H,m). MS TOF 466 (M+1⁺).
Hplc (Magellan C8, Gradient 3, water/acetonitrile/TFA)
30 rt 10.65 min.

Example 11**1-Aminoisoquinolin-7-oyl-D-phenylglycine-4-(3-pyridyl) piperazinamide**

35

1H nmr (CD₃CN) 8.75 (1H,s); 8.15 (2H,m); 7.90 (1H, d);
7.80 (2H, m); 7.65 (1H, m); 7.40 (3H, m); 7.25 (3H, m);

- 35 -

7.10 (1H,d); 6.05 (1H, s); 3.70 (3H, m); 3.30 (4H,m);
2.80 (1H,m). MS TOF 467 (M+1⁺). Hplc (Magellan C8,
Gradient 3, water/acetonitrile/TFA) rt 8.30 min.

5 **Example 12**

**1-Aminoisoquinolin-7-oyl-D-phenylglycine-4-(2-pyridyl)
piperazinamide**

1H nmr (CD₃CN) 8.75 (1H,s); 8.30 (1H,d); 8.20 (1H, d);
10 8.10 (1H, d); 7.90 (2H, m); 7.60 (3H, m); 7.45 (3H, m);
7.20 (1H,d); 7.10 (1H,d); 6.90 (1H,m); 6.20 (1H, s);
3.80 (3H, m); 3.55 (4H,m); 3.25 (1H,m). MS TOF 467
(M+1⁺). Hplc (Magellan C8, Gradient 3,
water/acetonitrile/TFA) rt 8.68 min.

15

Example 13

**1-Aminoisoquinolin-7-oyl-D-phenylglycine-4-(3-
trifluorophenyl) piperazinamide**

20 1H nmr (CD₃OD) 8.85 (1H,s); 8.25 (1H,d); 7.90 (1H, d);
7.45 (3H, m); 7.30 (4H, m); 7.15 (1H, d); 7.00 (3H, m);
6.15 (1H, s); 3.80 (1H, m); 3.65 (2H,m); 3.50 (1H,m);
3.15 (2H,m); 3.05 (1H,m); 2.65 (1H,m). MS TOF 534
(M+1⁺). Hplc (Magellan C8, Gradient 3,
25 water/acetonitrile/TFA) rt 12.72 min.

Example 14

**1-Aminoisoquinolin-7-oyl-D-phenylglycine-4-(2-
methoxyphenyl) piperazinamide**

30

1H nmr (CD₃CN) 8.85 (1H,s); 8.25 (1H,d); 7.85 (1H, d);
7.50 (1H, d); 7.45 (2H, m); 7.30 (3H, m); 7.15 (1H,d);
6.95 (1H,m); 6.85 (1H,d); 6.80 (2H,m); 6.10 (1H, s);
3.70 (3H, m); 3.50 (1H,m); 3.00 (3H,m); 2.45 (1H,m). MS
35 TOF 496 (M+1⁺). Hplc (Magellan C8, Gradient 3,
water/acetonitrile/TFA) rt 9.55 min.

- 36 -

Example 15**1-Aminoisoquinolin-7-oyl-D-phenylglycine-4-(2-methylphenyl) piperazinamide**

5 1H nmr (CD₃CN) 8.85 (1H,s); 8.25 (1H,d); 7.85 (1H, d);
7.55 (1H, d); 7.45 (2H, m); 7.35 (3H, m); 7.15 (1H,d);
6.95 (2H,m); 6.80 (2H,m); 6.10 (1H, s); 3.80 (1H, m);
3.60 (2H,m); 3.45 (1H,m); 2.75 (3H,m); 2.30 (1H,m). MS
TOF 480 (M+1⁺). Hplc (Magellan C8, Gradient 3,
10 water/acetonitrile/TFA) rt 9.75 min.

Example 16**1-Aminoisoquinolin-7-oyl-D-phenylglycine-4-(-3,4-methylenedioxybenzyl) piperazinamide**

15 1H nmr (CD₃CN) 8.55 (1H,s); 8.15 (1H,d); 7.70 (1H, d);
7.40 (1H, d); 7.30 (5H, m); 7.00 (1H,d); 6.75 (1H,s);
6.70 (2H,s); 5.85 (1H, s); 3.90 (2H, s); 3.60 (2H,m);
3.00-2.00 (broad signal 8H). MS TOF 524 (M+1⁺). Hplc
20 (Magellan C8, Gradient 3, water/acetonitrile/TFA) rt
9.25 min.

Example 17**1-Aminoisoquinolin-7-oyl-D-phenylglycine-4-(4'-fluorophenyl) piperazinamide**

25 1H nmr (CD₃CN) 8.75 (1H,s); 8.20 (1H,d); 7.85 (1H, d);
7.55 (1H, d); 7.40 (5H, m); 7.15 (1H,d); 7.05 (4H,m);
6.05 (1H, s); 3.80 (3H, m, obscured by solvent); 3.55
30 (1H,m); 3.25 (2H,m); 3.10 (1H,m); 2.60 (1H,m). MS TOF
484 (M+1⁺). Hplc (Magellan C8, Gradient 3,
water/acetonitrile/TFA) rt 11.31 min.

Example 18

1-Aminoisoquinolin-7-oyl-D-phenylglycine-4-(2-chlorophenyl) piperazinamide

5 1H nmr (CD₃CN) 8.85 (1H,s); 8.45 (1H,d); 8.10 (1H, d);
7.75 (1H, d); 7.50 (6H, m); 7.40 (2H,m); 7.20 (2H,m);
6.30 (1H, s); 4.00 (1H,m); 3.85 (2H,m); 3.70 (1H,m);
3.25 (1H,m); 3.10 (2H,m); 2.60 (1H,m). MS TOF 501
(M+1⁺). Hplc (Magellan C8, Gradient 3,
10 water/acetonitrile/TFA) rt 12.66 min.

Example 19

1-Aminoisoquinolin-7-oyl-D-phenylglycine-4-(2-pyrimidyl) piperazinamide

15 1H nmr (CD₃CN) 8.85 (1H,s); 8.35 (2H,d); 8.25 (1H,d);
7.90 (1H, d); 7.60 (1H, d); 7.45 (5H, m); 7.20 (1H,d);
6.65 (1H,t); 6.10 (1H, s); 3.90-3.50 (broad signal 6H);
3.50 (1H,m); 3.20 (1H,m). MS TOF 468 (M+1⁺). Hplc
20 (Magellan C8, Gradient 3, water/acetonitrile/TFA) rt
9.22 min.

Example 20

1-Aminoisoquinolin-7-oyl-D-phenylglycine piperidinamide

25 1H nmr (CD₃CN) 9.10 (1H,s); 8.80 (1H,d); 8.35 (1H,d);
8.00 (1H,d); 7.70 (1H, d); 7.50 (2H, d); 7.40 (5H, m);
7.25 (1H,d); 6.15 (1H, s); 3.70-3.30 (broad 4H,m); 1.60-
1.00 (broad 6H, m). MS TOF 389 (M+1⁺). Hplc (Magellan
30 C8, Gradient 3, water/acetonitrile/TFA) rt 10.47 min.

Example 21

1-Aminoisoquinolin-7-oyl-D-phenylglycine-4-(4'-pyridyl) piperidinamide

35 1H nmr (CDCl₃) 9.05 (1H,s); 8.80 (1H,d); 8.60 (2H,m);
8.35 (1H,d); 8.00 (1H,d); 7.70 (1H, d); 7.55 (3H, m);

- 38 -

7.40 (5H, m); 7.25 (1H,d); 6.20 (1H, s); 4.70 (1H,m);
4.30-1.50 (broad 9H,m). MS TOF 466 (M+1⁺). Hplc
(Magellan C8, Gradient 3, water/acetonitrile/TFA) rt
9.58 min.

5

Example 22

**1-Aminoisoquinolin-7-oyl-D-phenylglycine-4-
phenoxypiperidinamide**

10 1H nmr (DMSO) 9.05 (1H,s); 8.40 (1H,d); 8.05 (2H,m);
7.65 (1H,d); 7.55 (2H,m); 7.40 (3H, m); 7.25 (3H, m);
6.90 (3H, m); 6.10 (1H, s); 4.55 (1H,m); 3.85 (2H,m);
3.35 (2H,m); 1.90 (1H,m); 1.60 (1H,m); 1.30 (1H,m); 0.90
15 (1H,m). MS TOF 481 (M+1⁺). Hplc (Magellan C8, Gradient
3, water/acetonitrile/TFA) rt 11.95 min.

Example 23

**1-Aminoisoquinolin-7-oyl-D-phenylglycine-2(RS)-
methylcyclohexamide**

20

1H nmr (DMSO) Mixture of diastereomers (major reported)
9.00 (1H,s); 8.25 (1H,d); 7.95 (1H,d); 7.60 (1H,d); 7.45
(2H, m); 7.25 (4H, m); 5.60 (1H, s); 3.10 (1H,m); 1.80-
0.80 (broad m, 9H) 0.40 (3H,m). MS TOF 417 (M+1⁺).
25 Hplc (Magellan C8, Gradient 3, water/acetonitrile/TFA)
rt 11.23 min.

Example 24

**1-Aminoisoquinolin-7-oyl-D-phenylglycine-1-S-
cyclohexylethylamide**

30

1H nmr (CD₃CN) 8.80 (1H,s); 8.25 (1H,d); 7.90 (1H,d);
7.50 (1H,d); 7.45 (2H, m); 7.30 (4H, m); 7.15 (1H,d);
5.35 (1H, s); 3.60 (1H,m); 1.70-0.50 (broad m, 11H) 1.00
35 (3H,d). MS TOF 431 (M+1⁺). Hplc (Magellan C8, Gradient
3, water/acetonitrile/TFA) rt 11.09 min.

Example 25**1-Aminoisoquinolin-7-oyl-D-phenylglycine-4-methoxybenzylamide**

5 1H nmr (CD₃CN) 8.60 (1H,s); 8.05 (1H,d); 7.70 (1H,d);
7.35 (1H,d); 7.30 (2H, m); 7.15 (3H, m); 7.00 (1H,d);
6.85 (2H,d); 6.60 (2H,d); 5.45 (1H, s); 4.10 (2H,m). MS
TOF 441 (M+1⁺). Hplc (Magellan C8, Gradient 3,
water/acetonitrile/TFA) rt 10.91 min.

10

Example 26**1-Aminoisoquinolin-7-oyl-D-phenylglycine-3, 4-methylenedioxybenzylamide**

15 1H nmr (CD₃CN) 8.65 (1H,s); 8.25 (1H,d); 7.85 (1H,d);
7.45 (3H,m); 7.30 (3H, m); 7.15 (1H,d); 6.65 (3H,m);
5.55 (1H, s); 4.20 (2H,m). MS TOF 455 (M+1⁺). Hplc
(Magellan C8, Gradient 3, water/acetonitrile/TFA) rt
11.02 min.

20

Example 27**1-Aminoisoquinolin-7-oyl-D-phenylglycine-1-naphthylamide**

25 1H nmr (CD₃CN) 8.45 (1H,s); 8.20 (1H,d); 7.80 (1H,d);
7.65 (1H,d); 7.60 (2H,m); 7.50 (1H,m); 7.4-7.0 (9H,m);
6.80 (1H,d); 5.80 (1H, s); 4.60 (2H,m). MS TOF 461
(M+1⁺). Hplc (Magellan C8, Gradient 3,
water/acetonitrile/TFA) rt 11.61 min.

30

Example 28**1-Aminoisoquinolin-7-oyl-D-phenylglycine-1,2,3,4-tetrahydro-1-naphthylamide**

35 1H nmr (CD₃CN) 8.75 (1H,s); 8.30 (1H,d); 7.95 (1H,d);
7.65 (2H,d); 7.40 (3H,m); 7.30 (1H,d); 7.10 (5H,m); 5.65
(1H, s); 5.10 (1H,m); 2.75 (2H,m) 2.4-1.5 (4H, broad
m). MS TOF 451 (M+1⁺). Hplc (Magellan C8, Gradient 3,

- 40 -

water/acetonitrile/TFA) rt 11.60 min.

Example 29**1-Aminoisoquinolin-7-oyl-D-phenylglycine-1-indanamide**

5
1H nmr (CD₃CN) 8.75 (1H,s); 8.30 (1H,d); 7.95 (1H,d);
7.55 (3H,m); 7.40 (3H,m); 7.30 (1H,d); 7.20 (4H,m); 5.65
(1H, s); 5.40 (1H,m); 2.90 (2H,m) 2.50 (1H,m); 1.80
(1H,m). MS TOF 437 (M+1⁺). Hplc (Magellan C8, Gradient
10 3, water/acetonitrile/TFA) rt 11.26 min.

Example 30**1-Aminoisoquinolin-7-oyl-D-phenylglycine-1, 2,3,4-tetrahydroisoquinolinamide**

15
1H nmr (DMSO) 9.20 (1H,s); 8.55 (1H,d); 8.20 (1H,d);
7.95 (1H,d); 7.70 (2H,m); 7.55 (3H,m); 7.40 (1H,d); 7.25
(4H,m); 6.40 (1H, s); 4.80 (2H,m); 3.90 (2H,m) 3.90
(2H,m). MS TOF 437 (M+1⁺). Hplc (Magellan C8, Gradient
20 3, water/acetonitrile/TFA) rt 11.34 min.

Example 31**1-Aminoisoquinolin-7-oyl-D-phenylglycine-{3-(3-aminophenyl)methyl}anilide**

25
1H nmr (DMSO) 9.00 (1H,s); 8.30 (1H,d); 7.95 (1H,d);
7.65 (1H,d); 7.50 (2H,m); 7.30 (4H,m); 7.15 (4H,m); 6.75
(4H,m); 5.75 (1H, s); 3.80 (2H,s). MS TOF 502 (M+1⁺).
Hplc (Magellan C8, Gradient 3, water/acetonitrile/TFA)
30 rt 9.48 min.

Example 32**1-Aminoisoquinolin-7-oyl-D-phenylglycine-3-acetylphenylanilide**

35
1H nmr (DMSO) 8.85 (1H,s); 8.25 (1H,s); 8.10 (1H,d);
7.85 (2H,m); 7.75 (1H,d); 7.65 (3H,m); 7.40 (3H,m);

- 41 -

6.95 (2H,m); 5.95 (1H, s); 2.50 (3H,s). MS TOF 439 (M+1+). Hplc (Magellan C8, Gradient 3, water/acetonitrile/TFA) rt 10.55 min.

5 **Example 33**

1-Aminoisoquinolin-7-oyl-D-phenylglycine-3(-1-hydroxyethyl)phenylanilide

1H nmr (DMSO) 8.95 (1H,s); 8.15 (1H,s); 7.95 (1H,d);
10 7.80 (1H,d); 7.65 (2H,m); 7.55 (1H,d); 7.45 (3H,m); 7.30 (1H,d); 7.05 (3H,m); 5.95 (1H, s); 4.75 (1H,m); 1.40 (3H,d). MS TOF 441 (M+1+). Hplc (Magellan C8, Gradient 3, water/acetonitrile/TFA) rt 10.03 min.

15 **Example 34**

1-Aminoisoquinolin-7-oyl-D-phenylglycine-4-(4'-chlorophenyl) piperazinamide

1H nmr (CD₃CN) 8.85 (1H,s); 8.30 (1H,d); 7.95 (1H, d);
20 7.65 (1H, d); 7.65 (2H,d); 7.50 (3H, m); 7.30 (3H,m); 7.00 (2H,d); 6.15 (1H, s); 3.95 (1H,m); 3.70 (3H, m); 3.25 (2H,m); 3.05 (1H,m); 2.55 (1H,m). MS TOF 501 (M+1⁺). Hplc (Magellan C8, Gradient 3, water/acetonitrile/TFA) rt 11.95 min.

25

Example 35

1-Aminoisoquinolin-7-oyl-D-phenylglycine-4-(4'-hydroxyphenyl) piperazinamide

30 1H nmr (CD₃CN) 8.85 (1H,s); 8.25 (1H,d); 7.95 (1H, d); 7.55 (3H, m); 7.45 (3H, m); 7.25 (3H,m); 6.90 (2H,d); 6.15 (1H, s); 3.95 (3H, m); 3.65 (1H,m, obscured by solvent); 3.40 (3H,m); 2.90 (1H,m). MS TOF 481 (M+1⁺). Hplc (Magellan C8, Gradient 3, water/acetonitrile/TFA)
35 rt 7.84 min.

Example 36

1-Aminoisoquinolin-7-oyl-D-phenylglycine-4-(4'-nitrophenyl) piperazinamide

5 1H nmr (CD₃CN) 8.70 (1H,s); 8.15 (1H,d); 7.95 (2H,d);
8.80 (1H, d); 7.50 (3H, m); 7.30 (3H, m); 7.05 (1H,d);
6.70 (2H,d); 6.00 (1H, s); 3.60 (3H, m); 3.30 (4H,m);
2.80 (1H,m). MS TOF 511 (M+1⁺). Hplc (Magellan C8,
Gradient 3, water/acetonitrile/TFA) rt 10.81min.

10

Example 37

1-Aminoisoquinolin-7-oyl-D-phenylglycine-4-(4'-methylphenyl) piperazinamide

15 1H nmr (CD₃CN) 8.85 (1H,s); 8.45 (1H,d); 8.05 (1H, d);
7.70 (3H, m); 7.55 (3H, m); 7.35 (1H,d); 7.25 (2H,d);
7.00 (2H,d); 6.30 (1H, s); 3.95 (3H, m); 3.70 (1H,m);
3.25 (3H,m); 2.60 (1H,m); 2.40 (3H,s). MS TOF 480
(M+1⁺). Hplc (Magellan C8, Gradient 3,
20 water/acetonitrile/TFA) rt 10.27 min.

Example 38

1-Aminoisoquinolin-7-oyl-D-phenylglycine-4-(4'-methoxyphenyl) piperazinamide

25

1H nmr (CD₃CN) 8.85 (1H,s); 8.25 (1H,d); 7.95 (1H, d);
7.55 (3H, m); 7.40 (3H, m); 7.20 (1H,d); 7.15 (2H,d);
6.95 (2H,d); 6.20 (1H, s); 3.85(3H,m); 3.75 (3H,s); 3.60
(1H,m); 3.30 (2H,m); 3.15 (1H,m); 2.75 (1H,m). MS TOF
30 496 (M+1⁺). Hplc (Magellan C8, Gradient 3,
water/acetonitrile/TFA) rt 8.86 min.

Example 39

1-Aminoisoquinolin-7-oyl-D,L-4-fluorophenylglycine-4-(4'-fluorophenyl) piperazinamide

35

1H nmr (DMSO) 9.05 (1H,s); 8.35 (1H,d); 8.05 (1H, d);

- 43 -

7.70 (1H, d); 7.55 (2H, m); 7.25 (3H, m); 7.05 (2H, m);
6.90 (2H, m); 6.15 (1H, s); 3.60 (3H, m); 3.05 (3H, m);
2.90 (1H, m); 2.60 (1H, m). MS TOF 502 (M+1+). Hplc
(Magellan C8, Gradient 3, water/acetonitrile/TFA) rt
5 11.13 min.

Example 40

**1-Aminoisoquinolin-7-oyl-D-cyclohexylglycine-4-(4'-
fluorophenyl) piperazinamide**

10

1H nmr (DMSO) 9.05 (1H, s); 8.70 (1H, d); 8.05 (1H, d);
7.70 (1H, d); 7.30 (1H, d); 7.00 (4H, m); 4.90 (1H, m);
3.85 (2H, m); 3.70 (2H, m); 3.05 (4H, m); 1.90 (1H, m);
1.70 (4H, m); 1.15 (6H, m). MS TOF 490 (M+1+). Hplc
15 (Magellan C8, Gradient 3, water/acetonitrile/TFA) rt
11.19 min.

Example 41

**1-Aminoisoquinolin-7-oyl-D-phenylglycine-4-(3-
chlorophenyl) piperazinamide**

20

1H nmr (CD₃CN) 8.80 (1H, s); 8.25 (1H, d); 7.90 (1H, d);
7.50 (3H, m); 7.40 (3H, m); 7.20 (2H, m); 6.85 (3H, m);
6.15 (1H, s); 3.80 (1H, m); 3.65 (2H, m); 3.55 (1H, m);
25 3.20 (2H, m); 3.05 (1H, m); 2.55 (1H, m). MS TOF 501
(M+1⁺). Hplc (Magellan C8, Gradient 3,
water/acetonitrile/TFA) rt 11.47 min.

Example 42

**1-Aminoisoquinolin-7-oyl-D-phenylglycine-4-(3-
methylphenyl) piperazinamide**

30

1H nmr (CD₃CN) 8.80 (1H, s); 8.25 (1H, d); 7.95 (1H, d);
7.55 (3H, m); 7.45 (3H, m); 7.25 (2H, m); 6.95 (3H, m);
35 6.15 (1H, s); 3.95 (1H, m); 3.75 (2H, m); 3.65 (1H, m);
3.30 (2H, m); 3.15 (1H, m); 2.60 (1H, m); 2.30 (3H, s). MS
TOF 480 (M+1⁺). Hplc (Magellan C8, Gradient 3,

- 44 -

water/acetonitrile/TFA) rt 10.20 min.

Example 43

5 **1-Aminoisoquinolin-7-oyl-D-phenylglycine-4-(3-methoxyphenyl) piperazinamide**

1H nmr (CD₃CN) 8.80 (1H,s); 8.20 (1H,d); 7.85 (1H, d);
7.45 (1H,d); 7.40 (2H, m); 7.25 (3H, m); 7.05 (1H,d);
6.95 (1H,m); 6.35 (1H,d); 6.30 (2H, m); 6.10 (1H, s);
10 3.80 (1H, m); 3.60 (2H,m); 3.55 (3H,s); 3.45 (1H,m);
3.05 (2H,m); 2.85 (1H,m); 2.45 (1H,m). MS TOF 496
(M+1⁺). Hplc (Magellan C8, Gradient 3,
water/acetonitrile/TFA) rt 10.85 min.

15 **Example 44**

1-Aminoisoquinolin-7-oyl-D-phenylglycine-4-(2-fluorophenyl) piperazinamide

1H nmr (CD₃CN) 8.80 (1H,s); 8.25 (1H,d); 7.95 (1H, d);
20 7.50 (6H, m); 7.20 (2H,d); 7.00 (4H,m); 6.15 (1H, s);
3.05 (6H,m); 2.45 (1H,m); 2.15 (1H,m). MS TOF 484
(M+1⁺). Hplc (Magellan C8, Gradient 3,
water/acetonitrile/TFA) rt 11.06 min.

25 **Example 45**

1-Aminoisoquinolin-7-oyl-D-phenylglycine-4-(2-hydroxyphenyl) piperazinamide

1H nmr (CD₃CN) 8.85 (1H,s); 8.25 (1H,d); 7.95 (1H, d);
30 7.50 (6H, m); 7.25 (3H,m); 7.00 (2H,m); 6.15 (1H, s);
4.00 (1H,m); 3.80 (2H,m); 3.70 (1H,m); 3.35 (3H,m); 2.80
(1H,m). MS TOF 482 (M+1⁺). Hplc (Magellan C8, Gradient
3, water/acetonitrile/TFA) rt 8.44 min.

35

Example 46

1-Aminoisoquinolin-7-oyl-D-phenylglycine-4-(2,4-difluorophenyl) piperazinamide

5 1H nmr (CD₃CN) 8.85 (1H,s); 8.25 (1H,d); 7.95 (1H, d);
7.50 (6H, m); 7.25 (1H,d); 6.95 (3H,m); 6.15 (1H, s);
3.85 (1H,m); 3.65 (2H,m); 3.50 (1H,m); 2.95 (3H,m); 2.40
(1H,m). MS TOF 502 (M+1⁺). Hplc (Magellan C8, Gradient
3, water/acetonitrile/TFA) rt 11.27 min.

10

Example 47

1-Aminoisoquinolin-7-oyl-D-phenylglycine-4-(2,3-dimethylphenyl) piperazinamide

15 1H nmr (CD₃OH) 8.60 (1H,s); 8.05 (1H,d); 7.65 (1H, d);
7.20 (6H, m); 6.95 (1H,d); 6.65 (1H,m); 6.55 (1H,d);
6.45 (1H,d); 5.95 (1H, s); 3.60 (1H,m); 3.50 (2H,m);
3.30 (1H,m); 2.55 (3H,m); 2.10 (1H,m) 1.90 (6H,d). MS
TOF 494 (M+1⁺). Hplc (Magellan C8, Gradient 3,
20 water/acetonitrile/TFA) rt 11.98 min.

Example 48

1-Aminoisoquinolin-7-oyl-D-phenylglycine-4-(2,4-dimethylphenyl) piperazinamide

25

1H nmr (CD₃OH) 8.65 (1H,s); 8.05 (1H,d); 7.65 (1H, d);
7.35 (1H,d); 7.20 (5H, m); 6.95 (1H,d); 6.65 (1H,s);
6.60 (1H,d); 6.50 (1H,d); 5.95 (1H, s); 3.60 (1H,m);
3.50 (2H,m); 3.30 (1H,m); 2.55 (3H,m); 2.10 (1H,m) 1.90
30 (6H,d). MS TOF 494 (M+1⁺). Hplc (Magellan C8, Gradient
3, water/acetonitrile/TFA) rt 12.00 min.

Example 49

35 **1-Aminoisoquinolin-7-oyl-D-phenylglycine-4-(2,6-dimethylphenyl) piperazinamide**

1H nmr (CD₃OH) 8.70 (1H,s); 8.10 (1H,d); 7.85 (1H, d);

- 46 -

7.30 (3H,m); 7.20 (3H, m); 7.00 (1H,d); 6.65 (3H,m);
5.95 (1H, s); 3.85 (1H,m); 3.40 (3H,m); 2.800 (2H,m);
2.65 (1H,m); 2.30 (1H,m) 2.10 (3H,s); 1.90 (3H,s). MS
TOF 494 (M+1⁺). Hplc (Magellan C8, Gradient 3,
5 water/acetonitrile/TFA) rt 12.04 min.

Example 50

**1-Aminoisoquinolin-7-oyl-D-phenylglycine-4-(2,5-
dimethylphenyl) piperazinamide**

10

1H nmr (CD₃OH) 8.65 (1H,s); 8.05 (1H,d); 7.65 (1H, d);
7.35 (1H,d); 7.25 (2H, m); 7.15 (3H,m); 6.95 (1H,d);
6.70 (1H,d); 6.50 (1H,d); 6.45 (1H,s); 5.95 (1H, s);
3.60 (1H,m); 3.50 (2H,m); 3.30 (1H,m); 2.55 (3H,m); 2.10
15 (1H,m) 1.95 (6H,d). MS TOF 494 (M+1⁺). Hplc (Magellan
C8, Gradient 3, water/acetonitrile/TFA) rt 12.09 min.

Example 51

**1-Aminoisoquinolin-7-oyl-D-cyclohexylglycine-4-(4'-
pyridyl) piperazinamide**

20

1H nmr (DMSO) 9.05 (1H,s); 8.35(1H,d); 8.25 (2H,d); 8.05
(1H,d); 7.75 (1H,d); 7.25 (1H, d); 7.15 (2H,d); 4.80
(1H, m); 4.10-3.50 (broad 8H,m, obscured by solvent);
25 1.95 (2H,m); 1.70 (4H,m); 1.20 (5H,m). MS TOF 473
(M+1⁺). Hplc (Magellan C8, Gradient 3,
water/acetonitrile/TFA) rt 8.00 min.

Example 52

**1-Aminoisoquinolin-7-oyl-D-phenylglycine-4-phenyl
piperidinamide**

30

1H nmr (CD₃CN) 8.90 (1H,s); 8.35 (1H,d); 8.05 (1H, d);
7.70-7.20 (11H, m); 7.10 (1H, d); 6.20 (1H, s); 4.70
35 (1H, m); 3.30 (1H,m); 2.80 (2H,m); 1.80 (2H,m); 1.50
(2H,m); 0.50 (1H,m). MS TOF 465 (M+1⁺). Hplc (Magellan
C8, Gradient 3, water/acetonitrile/TFA) rt 11.77 min.

Example 53**1-Aminoisoquinolin-7-oyl-D-phenylglycine-4-benzyl
piperidinamide**

5 1H nmr (CD₃CN) mixture of rotomers 8.65 (1H,d); 8.25
 (1H,m); 7.80 (1H, m); 7.55-7.00 (12H, m); 6.10 (1H, m);
 4.50 (1H, m); 3.90 (1H,m); 2.80 (4H,m); 1.60 (2H,m);
 1.25 (2H,m); 1.00 (1H,m); 0.25 (1H,m). MS TOF 479
 (M+1⁺). Hplc (Magellan C8, Gradient 3,
10 water/acetonitrile/TFA) rt 12.66 min.

Example 54**1-Aminoisoquinolin-7-oyl-D-phenylglycine-4-
piperidinopiperidinamide**

15 1H nmr (CD₃CN) mixture of confomers 8.70 (1H,d);
 8.25 (1H,m); 8.10 (1H,m); 7.75 (1H,m); 7.45 (5H,m); 7.05
 (1H, d); 6.10 (1H, m); 4.70 (1H,m); 4.10 (1H,m); 3.50-
 3.00 (broad 3H,m); 3.00-2.30 (broad 7H,m); 2.10 (1H,m);
20 1.80 (5H,m); 1.40 (1H,m). MS TOF 472 (M+1⁺). Hplc
 (Magellan C8, Gradient 2, water/acetonitrile/TFA) rt
 12.09 min.

Example 55**25 1-Aminoisoquinolin-7-oyl-D-cyclohexylglycine-1-S-
 cyclohexylethylamide**

 1H nmr (DMSO) 9.05 (1H,s); 8.35 (1H,d); 8.05 (1H,d); 7.75
 (1H,d); 7.25 (1H, d); 4.40 (1H, m); 3.65 (1H,m); 1.85-
30 1.50 (broad 11H,m); 1.35-0.80 (broad 11H,m); 1.05
 (3H,d); MS TOF 423 (M+1⁺). Hplc (Magellan C8, Gradient
 3, water/acetonitrile/TFA) rt 11.68 min.

Method 5

35

By solid phase: Typically a Wang resin modified with a
4-nitrophenylcarbonate (see Kim, S.W., et al. Bioorg.

- 48 -

Med. Chem. Lett. 8 (1998) 735-738) is reacted with 4,4 bipiperidine The resin was further modified by the attachment of acids. Activation of Fmoc protected amino acid (2-5eq) was by TBTU/ DIPEA, all couplings (minimum 120 min.) were carried out in DMF. Deprotection of the Fmoc group was achieved with 20% piperidine in DMF. Other acid substituents were added as the HOBT or HOAt esters either by activation with HBTU/HATU or DIPCI with or without Boc protection of amino groups. Cleavage of the products from the resin was by treatment (30 min., ambient) with 10% triethylsilane in TFA, filtration, evaporation and trituration with diethylether.

Example 56

1-Aminoisoquinolin-7-oyl-D-phenylglycine-4,4-bipiperidinamide

Wang resin (1g, 1mmol./g) was swollen with dry DCM (25ml.) to this NMM (0.6ml., 5.6mmol.) and 4-nitrophenylchloroformate (400mg., 2mmol.) was added. The resin was agitated overnight then washed with DCM (25ml, x6) and DMF (25ml., x6). 4,4'Bipiperidine (332mg, 2mmol.) in DMF (20ml.) was then added and the resin agitated overnight. The resin was then washed with DMF (25ml., x6), DCM (25ml., x6) and diethyl ether (25ml., x3) then air dried.

The above modified resin (100mg., 0.1mmol.) was treated with Fmoc-D-Phenylglycine (0.5 mmol, 187mg), DMF(2.5ml), TBTU in DMF(1.25ml of a 450mM solution) and DIPEA in DMF (1.25ml of a 900 mM solution). The mixture was agitated with nitrogen for 2 hours. Deprotection and washing as Method 1.

A solution of 1-aminoisoquinoline-7-carboxylic acid trifluoroacetic acid salt (150mg 0.5mmole) (Example 88) in dry dimethylformamide (DMF) was treated successively

- 49 -

with HOAt (102mg 0.75mmole) and EDC (115mg 0.6mmole) and stirred at room temperature for 10min. The mixture was transferred to the reaction vessel on the Symphony and agitated for 10 hours with nitrogen. The resin was
5 washed with DMF (6x5ml), DCM (6x5ml) and air dried. The product was cleaved from the resin with 10% triethylsilane in TFA (10ml) for 30 minutes, the resin filtered off and the TFA solution evaporated to dryness and triturated with diethyl ether to give the crude
10 product. The crude product was dissolved in water (10ml), filtered and purified by preparative reverse phase Hplc.

1H nmr (DMSO) 8.80 (1H,s); 8.15 (1H,d); 7.70 (1H,d);
15 7.50 (1H,d); 7.25 (2H,m); 7.10 (3H, m); 7.00 (1H,d); 5.90 (1H, s); 4.30 (1H,m); 3.80 (1H,m); 3.3-2.3 (8H, various m, obscured by solvent); 1.80-0.50 (8H, various m). MS TOF 472 (M+1+). Hplc (Magellan C8, Gradient 1, water/acetonitrile/TFA) rt 7.32 min.

20

Other compounds made by the above method:

Example 57

1-Aminoisoquinolin-7-oyl-D,L-2-thienylglycine-4,4-
25 bipiperidinamide

MS TOF 478 (M+1+). Hplc (Magellan C8, Gradient 3, water/acetonitrile/TFA) rt 10.43 min.

30

Example 58

1-Aminoisoquinolin-7-oyl-D,L-1-naphthylglycine-4,4-
bipiperidinamide

35

MS TOF 522 (M+1+). Hplc (Magellan C8, Gradient 3, water/acetonitrile/TFA) rt 12.99 min.

- 50 -

Example 59

1-Aminoisoquinolin-7-oyl-D-cyclohexylglycine-4,4-bipiperidinamide

5 MS TOF 478 (M+1+). Hplc (Magellan C8, Gradient 3, water/acetonitrile/TFA) rt 12.65 min.

Example 60

10 **1-Aminoisoquinolin-7-oyl-L-phenylglycine-4,4-bipiperidinamide**

MS TOF 472 (M+1+). Hplc (Magellan C8, Gradient 3, water/acetonitrile/TFA) rt 10.89 min.

15 **Example 61**

1-Aminoisoquinolin-7-oyl-D,L-3-chlorophenylglycine-4,4-bipiperidinamide

20 MS TOF 507 (M+1+). Hplc (Magellan C8, Gradient 3, water/acetonitrile/TFA) rt 12.54 min.

Example 62

25 **1-Aminoisoquinolin-7-oyl-D-isoleucine-4,4-bipiperidinamide**

MS TOF 452 (M+1+). Hplc (Magellan C8, Gradient 3, water/acetonitrile/TFA) rt 11.04 min.

Method 6

30

Typically Boc-(R)-1,2 diamino-1-phenylethane was prepared by the method of O'Brien, P. et al. J. Med. Chem. 37 (1994) 12, 1810-1822. The free amino group was reacted with an acid chloride or an activated acid, all
35 couplings (minimum 120 min.) were carried out in DMF. After an aqueous work up the deprotection of the Boc group was achieved with TFA. Other acid substituents

- 51 -

were added as the HOBt or HOAt esters either by activation with HBTU/HATU, EDC or DIPCI with or without Boc protection of amino groups. The final products were purified by preparative reverse phase Hplc.

5

Example 63

2(R)-{[N-(1-Aminoisoquinoline)-7-oyl]amino}-2-phenylethyl-4-methylbenzamide

10 Boc-(R)-1,2 diamino-1-phenylethane (2g) was dissolved in DCM (80ml) and toluoylchloride (1.2ml.) and TEA (1.1ml.) added. The mixture was stirred at room temperature under argon overnight. The mixture was then washed with
15 2M sodium hydroxide solution, water, 5% hydrochloric acid and brine, dried over magnesium sulphate, filtered and evaporated. The product was recrystallised from hexane dissolved in DCM (50ml.) and treated with TFA for 2hrs. The solvent was evaporated and the solid
20 triturated with diethylether.

20

A solution of 1-aminoisoquinoline-7-carboxylic acid trifluoroacetic acid salt (300mg 1mmole) in dry DMF (10ml.) was treated successively with HOAt (204mg 1.5 mmole) and EDC (230mg 1.2mmole) and stirred at room
25 temperature for 10min. The mixture was added to the R-Amino-2-phenylethyl-4-methylbenzamide. TFA salt (0.38g, 1.03mmol.) with DIPEA (129mg, 1mmol.) and stirred overnight. After aqueous work up the final products were purified by preparative reverse phase Hplc.

30

1H nmr (DMSO) 8.90 (1H,s); 8.35 (1H,d); 7.95 (1H,d); 7.65 (1H,d); 7.60 (2H,d); 7.55 (1H,d); 7.25 (6H,m); 5.35 (1H, m); 3.65 (2H,m); 2.25 (3H,s). MS TOF 425 (M+1+). Hplc (Magellan C8, Gradient 3,
35 water/acetonitrile/TFA) rt 10.88 min.

Other compounds made by the above method:

Example 64

2(R)-{[N-(1-Aminoisoquinoline)-7-oyl]amino}-2-phenylethyl-4-methoxybenzamide

5 1H nmr (DMSO) 9.00 (1H,s); 8.50 (1H,d); 8.20 (1H,d);
7.85 (3H,m); 7.60 (1H,d); 7.45 (5H,m); 7.15 (2H,d);
5.55 (1H, m); 3.95 (3H,s); 3.85 (2H,m). MS TOF 441
(M+1+). Hplc (Magellan C8, Gradient 3,
water/acetonitrile/TFA) rt 10.90 min.

10

Example 65

2(R)-{[N-(1-Aminoisoquinoline)-7-oyl]amino}-2-phenylethyl benzamide

15 1H nmr (CD₃OD) 8.75 (1H,s); 8.25 (1H,d); 7.85 (1H,d);
7.60 (2H,d); 7.55 (1H,d); 7.30 (9H,m); 5.35 (1H, m);
3.85 (2H,m). MS TOF 410 (M+1⁺). Hplc (Magellan C8,
Gradient 3, water/acetonitrile/TFA) rt 10.26 min.

20

Example 66

2(R)-{[N-(1-Aminoisoquinoline)-7-oyl]amino}-2-phenylethyl benzylamide

25 1H nmr (CD₃OD) 8.65 (1H,s); 8.10 (1H,d); 7.80 (1H,d);
7.55 (1H,d); 7.55 (1H,d); 7.25 (7H,m); 7.05 (1H,d); 6.95
(3H,m); 5.25 (1H, m); 4.15 (1H,d); 3.75 (2H,m). MS TOF
424 (M+1⁺). Hplc (Magellan C8, Gradient 3,
water/acetonitrile/TFA) rt 10.13 min.

30

Example 67

2(R)-{[N-(1-Aminoisoquinoline)-7-oyl]amino}-2-phenylethyl benzosulphonamide

35 1H nmr (CD₃OD) 8.55 (1H,s); 7.95 (1H,d); 7.70 (4H,m);
7.45 (3H,m); 7.20 (5H,m); 6.90 (1H,d); 5.10 (1H, m);
3.30 (2H,m). MS TOF 447 (M+1⁺). Hplc (Magellan C8,
Gradient 3, water/acetonitrile/TFA) rt 11.22 min.

- 53 -

Example 68

2(R)-{[N-(1-Aminoisoquinoline)-7-oyl]amino}-2-phenylethyl cyclohexanecarboxamide

5 1H nmr (CD₃OD) 8.55 (1H,s); 7.95 (1H,d); 7.70 (2H,m);
7.25 (5H,m); 6.95 (1H,d); 5.20 (1H, m); 3.50 (2H,m) 2.05
(1H,m); 1.6-1.0 (10H, broad m). MS TOF 417 (M+1⁺). Hplc
(Magellan C8, Gradient 3, water/acetonitrile/TFA) rt
11.29 min.

10

Example 69

2(R)-{[N-(1-Aminoisoquinoline)-7-oyl]amino}-2-phenylethyl cyclohexyl acetamide

15 1H nmr (CD₃OD) 8.55 (1H,s); 7.95 (1H,d); 7.70 (2H,m);
7.25 (5H,m); 6.95 (1H,d); 5.20 (1H, m); 3.55 (2H,m) 2.00
(2H,m); 1.7-0.6 (11H, broad m). MS TOF 431 (M+1⁺). Hplc
(Magellan C8, Gradient 3, water/acetonitrile/TFA) rt
11.49 min.

20

Method 7

Typically 4,4'-Bipiperidine was mono protected with the
tert-butyl oxy carbonyl (Boc) group. The free amino
25 group was reacted with an alkylating agent. After an
aqueous work up the deprotection of the Boc group was
achieved with TFA. Other acid substituents were added
as the HOBT or HOAt esters either by activation with
HBTU/HATU, EDC or DIPCI with or without Boc protection
30 of amino groups. The final products were purified by
preparative reverse phase Hplc.

t-Butyl 4-(piperidin-4-yl)piperidine-1-carboxylate
4,4'-Bipiperidine dihydrochloride (5g, 20.7 mmol) was
35 dissolved in NaOH (30ml, 2N) and ethanol (50ml).
To this solution was added Di-tert-butyl dicarbonate
(4.52g, 20.7 mmol) and the reaction mixture left to stir

- 54 -

overnight. The ethanol was removed under *vacuo* and the basic layer extracted with ethyl acetate (100ml) to remove any DiBoc material. The desired product was then extracted into aq. 5% HCl and the pH adjusted to 11.

5 Extraction with ethyl acetate (100ml), washing with brine and drying gave the title compound as a white solid.

Example 70

10 **1-Aminoisoquinolin-7-oyl-D-phenylglycine-4,4'-(1'-ethoxycarbonylmethyl)bipiperidinamide**

To a suspension of t-Butyl 4-(piperidin-4-yl)piperidine-1-carboxylate (536mg, 2 mmol), and potassium carbonate

15 (580mg, 4.2 mmol) in acetone (25mL) was added ethyl bromoacetate (248 μ L, 2.2 mmol) and the reaction mixture left to stir at room temperature overnight. The acetone was removed and the residue treated with ethyl acetate, washed with water, dried with magnesium sulphate,

20 filtered and evaporated. The product was further elaborated as Method 4 after the removal of the Boc protecting group.

1H nmr (CD_3CN) 8.55 (1H,s); 8.15 (1H,d); 8.05 (1H,d);

25 7.65 (1H,d); 7.35 (2H,m); 7.20 (3H, m); 7.00 (1H,d); 5.90 (1H, s); 4.45 (1H,m); 4.10 (2H,m); 3.80 (1H,m); 3.70 (2H,m); 3.3-2.3 (8H, various m, obscured by solvent); 1.70-0.70 (8H, various m); 1.10 (3H,t). MS TOF 558 ($M+1^+$). Hplc (Magellan C8, Gradient 3,

30 water/acetonitrile/TFA) rt 8.49 min.

Other compounds made by the above method:

Example 71

1-Aminoisoquinolin-7-oyl-D-phenylglycine-4,4'-(1'-ethyl)bipiperidinamide

5 1H nmr (CD₃OH) 8.75 (1H,s); 8.15 (1H,d); 7.75 (1H,d);
7.45 (1H,d); 7.25 (5H,m); 7.05 (1H,d); 5.95 (1H, s);
4.45 (1H,m); 3.80 (1H,m); 3.70 (2H,m); 3.35 (2H,m); 2.90
(2H,m); 2.60 (4H,m); 1.85 (1H,m); 1.65 (2H,m); 1.40-
0.70 (7H, various m); 1.10 (3H,t). MS TOF 500 (M+1⁺).
10 Hplc (Magellan C8, Gradient 3, water/acetonitrile/TFA)
rt 7.93 min.

Example 72

15 **1-Aminoisoquinolin-7-oyl-D-phenylglycine-4,4'-(1'-propyl)bipiperidinamide**

1H nmr (CD₃OH) 8.60 (1H,s); 8.05 (1H,d); 7.80 (1H,d);
7.45 (1H,d); 7.35 (5H,m); 7.10 (1H,d); 5.95 (1H, s);
4.35 (1H,m); 3.85 (1H,m); 3.40 (3H,m); 3.05-2.55 (broad
20 6H,m); 1.90 (1H,m); 1.60 (5H,m); 1.25 (4H,m); 0.80
(4H,m). MS TOF 514 (M+1⁺). Hplc (Magellan C8, Gradient
3, water/acetonitrile/TFA) rt 8.20 min.

Example 73

25 **1-Aminoisoquinolin-7-oyl-D-phenylglycine-4,4'-(1'-carboxymethyl)bipiperidinamide**

1-Aminoisoquin-7-oyl-D-phenylglycine-4,4'-(1'-ethoxycarbonylmethyl)bipiperidinamide (40mg, 0.05 mmol)
30 was treated with sodium hydroxide 2N (100 µL, 4eq.) in
ethanol (5mL) overnight. Purification by preparative
HPLC gave the title compound as a white solid.

35 1H nmr (CD₃CN) 8.80 (1H,s); 8.35 (1H,d); 8.15 (1H,d);
7.70 (1H,d); 7.35 (2H,m); 7.20 (3H, m); 6.95 (1H,d);
5.90 (1H, s); 4.45 (1H,m); 3.80 (1H,m); 3.65 (2H,m);
3.40 (2H,m); 3.00-2.30 (5H, vaious m); 1.70-0.70 (9H,

- 56 -

various m). MS TOF 530 (M+1⁺). Hplc (Magellan C8, Gradient 3, water/acetonitrile/TFA) rt 7.88 min.

Example 74

5 **1-Aminoisoquinolin-7-oyl-D-phenylglycine-4,4'-(1'-amidomethyl)bipiperidinamide**

1-Aminoisoquin-7-oyl-D-phenylglycine-4,4'-(1'-ethoxymethyl)bipiperidinamide (40mg, 0.05 mmol) was
10 treated with excess ammonia gas in anhydrous ethanol (5mL) for 5 days. Purification by preparative HPLC gave the title compound as a white solid.

1H nmr (CD₃CN) 8.65 (1H,s); 8.15 (1H,d); 7.75 (1H,d);
15 7.40 (3H,m); 7.25 (3H, m); 7.05 (1H,d); 5.95 (1H, s); 4.45 (1H,m); 3.90 (1H,m); 3.65 (2H,m); 3.35 (2H,m); 3.00-0.80 (14H, various m, obscured by solvent). MS TOF 529 (M+1⁺). Hplc (Magellan C8, Gradient 3, water/acetonitrile/TFA) rt 7.73 min.

20

Example 75

1-Aminoisoquinolin-7-oyl-D-phenylglycine-4,4'-(1'-methyl)bipiperidinamide

25 **CBz-D-phenylglycine-4-[1-(Boc)piperidin-4-yl]piperidinamide**

To a solution of CBz-D-phenylglycine (877mg, 3.1 mmol) in anhydrous THF (40ml) at -25°C was added N-methylmorpholine (336μl, 3.1 mmol) and isobutyl
30 chloroformate (419μl, 3.1 mmol). The resulting suspension was left to stir for 5 min at this temperature and then the t-Butyl 4-(piperidin-4-yl)piperidine-1-carboxylate (825 mg, 3.1 mmol) in
35 anhydrous THF (5ml) was added in one portion at -25°C and the reaction mixture allowed to warm to room temperature over 1 hour. The N-methylmorpholine

- 57 -

hydrochloride was filtered off and the THF removed under vacuo. The resulting solid was treated with TFA/DCM (20ml, 1:1) and the reaction mixture left to stir for 2 hours. The organics were removed under vacuo, the residue dissolved in ethyl acetate (100 ml), washed with aq. sodium bicarbonate solution, brine, dried and concentrated to yield the title compound as a clear oil (1.12g, 84% over two steps).

10 **CBz-D-phenylglycine-4-(1-methylpiperidin-4-yl)piperidinamide**

To a solution of CBz-D-phenylglycine-4-[1-(Boc)piperidin-4-yl]piperidinamide (1.1g, 2.5 mmol) in acetonitrile (40ml) and aq. formaldehyde (10ml) was added sodium cyanoborohydride (315mg, 5 mmol) portionwise over 10 min. The reaction mixture was left to stir for 3 hours and then poured onto water (50ml), containing conc ammonia solution (5ml) and left to stir for 15 min. Extraction with ethyl acetate (100ml), washing with brine and drying gave the title compound as a colourless oil (1.1g, 97%).

25 **D-Phenylglycine-4-(1-methylpiperidin-4-yl)piperidinamide**

A vigorously stirred solution of CBz-D-phenylglycine-4-(1-methylpiperidin-4-yl)piperidinamide (1.1g, 2.45 mmol) in methanol (120ml), containing Palladium on carbon (200mg) was treated with hydrogen gas (balloon) at atmospheric pressure overnight. The reaction mixture was filtered through celite and concentrated to yield the title compound as a colourless oil (585mg, 76%).

35 The resultant product was coupled to 1-amino-isoquinoline carboxylic acid (TFA salt) using method 4.

¹H nmr (CD₃CN) 8.60 (1H,s); 8.10 (1H,d); 7.75 (1H,d);

- 58 -

7.45 (3H,m); 7.25 (3H, m); 7.00 (1H,d); 5.90 (1H, s);
4.30 (1H,m); 3.80 (1H,m); 3.3-2.3 (8H, various m,
obscured by solvent); 2.50 (3H,s); 1.80-0.50 (8H,
various m). MS TOF 486 (M+1⁺). Hplc (Magellan C8,
5 Gradient 3, water/acetonitrile/TFA) rt 9.11 min.

Example 76

**2(R)-{[N-(1-Aminoisoquinoline)-7-oyl]amino}-2-
phenylethyl-4-methylphenylcarbamate**

10

To a solution of Boc-(R)-phenylglycinol (500mg
3.65mmole) in DMF (20ml) was added 4-
methylphenylisocyanate (0.5 ml 4.0mmole) and the mixture
warmed to 50°C before standing at room temperature
15 overnight. Dilution with ethyl acetate and washing with
sat. sodium bicarbonate gave after drying (magnesium
sulphate) and evaporation of solvent the crude
intermediate which was purified by flash chromatography
(silica gel, EtOAC 0-20% in DCM)

20

Deprotection of the amine was carried out using 50% TFA
in DCM (30min), evaporation of solvent under reduced
pressure, dissolving in DCM, washing with sat sod.
bicarbonate, drying (magnesium sulphate) and
25 evaporation.

Coupling of the intermediate to 1-amino-isoquinoline
carboxylic acid (TFA salt) was carried out using the
standard method 4.

30

1H nmr (DMSO) 9.05 (1H,s); 8.40 (1H,d); 8.05 (1H,d);
7.70 (1H,d); 7.55-7.20 (7H,m); 7.05 (3H,m); 5.55 (1H,
m); 4.35 (2H,d); 2.25 (3H,s). MS TOF 441 (M+1⁺). Hplc
(Magellan C8, Gradient 3, water/acetonitrile/TFA) rt
35 11.60 min.

- 59 -

Example 77**1-Aminoisoquinolin-7-oyl-D-phenylglycine-2- (4-aminophenoxy)cyclopentyl amide**

5 Cyclopentene oxide (5g) was dissolved in ethanol (100ml) and water (25ml) and treated with sodium azide (3.9g) and ammonium chloride (3.2g) at reflux for 8h. the mixture was cooled, excess solvent evaporated off at reduced pressure and extracted with ether. The organic
10 solution was washed with water, dried (MgSO₄) and evaporated. The residues were taken up in hexane, washed with water to remove any remaining ethanol, dried (MgSO₄) and evaporated to give trans-1-azidocyclopentan-2-ol 3.6g.

15 Trans-1-azidocyclopentan-2-ol 1.27g was dissolved in DMF and treated with sodium hydride (400mg. 60%) at room temperature for 1h. 4-Fluoronitrobenzene (1.41g) was added and the mixture stirred at 50°C for 4h and then
20 left at room temperature overnight. The reaction was quenched with water and extracted with ether. The organic solution was washed with water, dried (MgSO₄) and evaporated. Purification by flash chromatography (SiO₂-20% DCM/hexane) gave trans-2-(4-nitrophenoxy)
25 cyclopentylazide. ¹H nmr (CDCl₃) 8.22 (2H, d); 6.98 (2H, d); 5.63 (1H, m); 4.07 (1H, m); 2.20 (2H, m); 1.87 (4H, m).

30 Hydrogenation in ethanol (30ml) using 5%Pd on C (100mg) gave cis-2-(4-aminophenoxy)cyclopentylamine.

Trans-2-(4-aminophenoxy)cyclopentylamine was coupled to Boc-D-phenylglycine using TBTU/DIPEA/DMF as method 4, the resultant product was deprotected (TFA/DCM) and
35 coupled to 1-amino-isoquinoline carboxylic acid (TFA salt) using method 4.

- 60 -

¹H nmr (CD₃CN) 8.55 (1H,s); 8.10 (1H,d); 7.70 (1H,d);
7.35 (1H,d); 7.25 (5H, m); 7.00 (1H, d); 6.55 (4H,s);
5.40 (1H, s); 4.30 (1H,m); 4.00 (1H,m); 2.00-100 (broad
6H, m). MS TOF 496 (M+1⁺). Hplc (Magellan C8, Gradient
5 3, water/acetonitrile/TFA) rt 9.26 min.

Example 78**1-Aminoisoquinolin-7-oyl-D-phenylglyciny-4-
phenoxyproline**

10

t-Butyldicarbonate (1.1g) and then sat. sodium
bicarbonate (20ml) were added to a suspension of trans-
Hyp-OBn (1.1g) in THF (25ml) and the mixture stirred for
30min. The mixture was diluted with ethyl acetate and
15 washed with water, dried (MgSO₄) and evaporated. Flash
chromatography (SiO₂ DCM then ether) gave Boc-Hyp-OBn
1.18g

20

DEAD (diethyl azadicarboxylate) (0.59ml) was added to a
mixture of Boc-Hyp-OBn (1.1g), triphenylphosphine (0.9g)
and phenol (0.32g) in THF 10ml over 30min. The mixture
was stirred overnight and triphenylphosphine (0.45g),
phenol (0.16g) and DEAD (0.3ml) were added and the
mixture stirred overnight before concentration. The
25 residue was taken up in ethyl acetate and washed with 2M
sodium hydroxide, saturated sodium bicarbonate, dried
(MgSO₄) and evaporated. Flash chromatography (SiO₂
hexane then 20% ethylacetate in hexane) gave Boc-cis-4-
phenoxyproline benzyl ester 496mg.

30

Boc-cis-4-phenoxyproline benzyl ester was deprotected
(TFA/DCM) and coupled to Boc-D-phenylglycine which was
deprotected (TFA/DCM) and coupled to 1-
aminoisoquinoline-7-carboxylic acid (TFA salt) as method
35 4. The product was hydrogenated in ethanol (5% Pd on C,
atmospheric pressure) for 4h. to give the title
compound.

- 61 -

¹H nmr (CD₃CN) 9.05 (1H,s); 8.35 (1H,d); 8.00 (1H,d); 7.70 (1H,d); 7.55 (1H,d); 7.35 (7H, m); 7.00 (1H, m); 6.85 (2H,d); 6.00 (1H, s); 5.05 (1H,m); 4.60 (1H,d); 3.80 (1H,m); 3.60 (1H,m); 2.50 (1H,m); 2.25 (1H,m). MS TOF 511 (M+1⁺). Hplc (Magellan C8, Gradient 3, water/acetonitrile/TFA) rt 10.36min.

Example 79

10 **1-Aminoisoquinolin-7-oyl-D-phenylglycine-4-(4'-pyrimidyl) piperazinamide**

A mixture of Boc-piperazine (2g), sodium bicarbonate (2g) and 2,4-dichloro-pyrimidine (1.5g) in ethanol (40ml)/water (20ml) was stirred at room temperature for 15 1h. Ethyl acetate (200ml) was added and the mixture washed twice with water, dried (MgSO₄) and evaporated. The product was crysrallised from DCM/ether, 2,52g.

20 The above product (1g) was dissolved in ethanol (30ml) and hydrogenated (5% Pd on C, 50psi) for 4h. to give Boc-4-(4'-pyrimidyl)-piperazine. ¹H nmr (CDCl₃) 8.53 (1H, s); 8.41 (1H, d); 6.42 (1H, d); 3.45 (8H, m); 1.35 (9H, s).

25 Boc-4-(4'-pyrimidyl)-piperazine was deprotected (TFA/DCM) and coupled to Boc-D-phenylglycine using method 4 to give Boc-D-phenylglycine-4-(4'-pyrimidyl)-piperazinamide which was deprotected (TFA/DCM) and coupled to 1-aminoisoquinoline-7-carboxylic acid (TFA salt) using method 4.

35 ¹H nmr (CD₃CN) 9.00 (1H,s); 8.75 (1H,s); 8.30 (1H,d); 8.20 (1H,d); 7.95 (1H, d); 7.70 (1H, d); 7.50 (2H, m); 7.35 (3H, m); 7.25 (1H,d); 7.10 (1H,d); 6.15 (1H, s); 3.70-3.30 (broad 8H,m). MS TOF 468 (M+1⁺). Hplc (Magellan C8, Gradient 1, water/acetonitrile/TFA) rt 9.09 min.

Example 80

(R)-1-(2-{1-Aminoisoquinolin-7-oyl}amino-2-phenylethyl)piperidin-2-one

5 (R)-1-(2-Butoxycarbonylamino-2-phenylethyl) piperidin-2-one

Boc-(R)-1,2 diamino-1-phenylethane (prepared by the method of O'Brien, P. et al. J. Med. Chem. 37 (1994) 12, 1810-1822) (0.3g, 1.27mmol.), methyl-5-bromovalerate
10 (0.18ml., 1.27mmol.) and DBU (0.19ml) were dissolved in absolute ethanol(10ml.) and stirred under reflux for 3 days. The ethanol was removed by evaporation and the residue taken up into ethylacetate, washed with 10%
15 hydrochloric acid and brine, dried over magnesium sulphate, filtered and evaporated to give the product. The above product was further elaborated by Method 4 to the title compound.

20 ¹H nmr (CD₃OD) 8.80 (1H,s); 8.20 (1H,d); 7.85 (1H, d); 7.55 (1H, d); 7.25 (6H, m); 5.45 (1H, m); 4.10 (2H,m); 3.40 (2H,m); 2.70 (4H,m); 2.25 (2H,m). MS TOF 389 (M+1⁺). Hplc (Magellan C8, Gradient 3, water/acetonitrile/TFA) rt 9.58 min.

25

Example 81

(R)-1-(2-{1-Aminoisoquinolin-7-oyl}amino-2-phenylethyl)-1,2 dihydro-1-oxo isoquinoline

30 (R)-1-(2-Butoxycarbonylamino-2-phenylethyl)-1,2 dihydro-1-oxo isoquinoline

Boc-(R)-1,2 diamino-1-phenylethane (prepared by the method of O'Brien, P. et al. J. Med. Chem. 37 (1994) 12, 1810-1822) (0.82g) was dissolved in toluene (50ml.).
35 Homophthalic anhydride (2.2g) was added followed by TEA (1.8ml.). The mixture was heated to 120oC under

- 63 -

nitrogen overnight. The reaction mixture was allowed to cool and washed with 2M sodium hydroxide solution, 10% hydrochloric acid and brine, dried over magnesium sulphate, filtered and evaporated. The residue was dissolved in methanol (17.5ml.) and DCM (35ml.) and sodium borohydride (70mg.) added as one portion. The reaction mixture was stirred under argon for 6 days. 50% Hydrochloric acid solution was then added and the reaction stirred for 1 hr. The solvents were then removed under vacuo and the residue taken up into ethylacetate (50ml.), washed with water and brine, dried over magnesium sulphate, filtered and evaporated to give the product.

The above product was further elaborated by Method 4 to the title compound.

¹H nmr (CDCl₃) 8.95 (1H,s); 8.40 (1H,d); 8.25 (2H,d); 7.75 (1H, d); 7.60 (1H,m); 7.50 (3H,m); 7.35 (1H, d); 7.20 (5H, m); 6.90 (1H,d); 6.50 (1H,d); 5.45 (1H, m); 4.75 (1H,m); 4.10 (1H,m). MS TOF 435 (M+1⁺). Hplc (Magellan C8, Gradient 3, water/acetonitrile/TFA) rt 10.10 min.

Example 82

N-{1(R)- Phenyl-2-(N-phthalimido)ethyl}-1-aminoisoquinolin-7-carboxamide

(R)-N-(2-Butoxycarbonylamino-2-phenylethyl)phthalimide

30

Boc-(R)-1,2 diamino-1-phenylethane (prepared by the method of O'Brien, P. et al. J. Med. Chem. 37 (1994) 12, 1810-1822) (0.3g, 1.27mmol.), phthalic anhydride (0.2g., 1.4mmol.) and TEA (0.1ml) were dissolved in chloroform (20ml.) and stirred under reflux for 2 days. The reaction mixture was then washed with 10% hydrochloric acid, 2M sodium hydroxide solution and brine, dried over

- 64 -

magnesium sulphate, filtered and evaporated to give the product. The above product was further elaborated by Method 4 to the title compound.

5 1H nmr (CD₃OD) 9.00 (1H,s); 8.40 (1H,d); 8.10 (1H, d);
7.90 (4H,m); 7.80 (1H, d); 7.55 (2H,d); 7.35 (4H, m);
5.65 (1H, m); 4.15 (2H,d). MS TOF 437 (M+1⁺). Hplc
(Magellan C8, Gradient 3, water/acetonitrile/TFA) rt
11.19 min.

10

Example 83

2(R)-{N-(1-Aminoisoquinolin-7-oyl)amino}-2-phenylethyl-4-methylbenzoate

15 Boc-(R)-Phenylglycinol (2g, 8.4mmol.) was dissolved in
DCM (50ml) and toluoylchloride (1.11ml.) and TEA
(1.1ml.) added. The mixture was stirred at room
temperature under argon overnight. The mixture was then
washed with 2M sodium hydroxide solution, water, 5%
20 hydrochloric acid and brine, dried over magnesium
sulphate, filtered and evaporated. The product was
recrystallised from hexane dissolved in DCM (50ml.) and
treated with TFA for 2hrs. The solvent was evaporated
and the solid triturated with diethylether.

25 A solution of 1-aminoisoquinoline-7-carboxylic acid
trifluoroacetic acid salt (200mg 0.66mmole) in dry DMF
(5ml.) was treated successively with HOAt (140mg 1mmole)
and EDC (147mg 0.8mmole) and stirred at room temperature
for 10min. The mixture was added to the R-Amino-2-
30 phenylethyl-4-methylbenzoate. TFA salt (0.23g,
1.03mmol.) with DBU (0.2ml) and stirred overnight. After
aqueous work up the final products were purified by
preparative reverse phase Hplc.

35 1H nmr (DMSO) 8.90 (1H,s); 8.20 (1H,d); 7.90 (1H,d);
7.65 (2H,d); 7.60 (1H,d); 7.45 (2H,d); 7.20 (6H,m);
5.45 (1H, m); 4.45 (2H,d); 2.25 (3H,s). MS TOF 416

- 65 -

(M+1+). Hplc (Magellan C8, Gradient 3, water/acetonitrile/TFA) rt 11.82 min.

Example 84

5 **O-(1-Aminoisoquinolin-7-oyl)-R-mandelyl-4-methylbenzylamide**

R-Mandelic acid (152 mg, 1 mmol.) was dissolved in DMF(3ml) with HATU (380 mg., 1 mmol.) and DIPEA(350μl),
10 2 mmol.). To this mixture was added 4-methylbenzylamine (121 mg., 1 mmol.). The mixture was stirred overnight. The mixture was then taken up into ethylacetate and washed with water, sodium carbonate solution, water, 10% hydrochloric acid solution and water. The ethylacetate
15 was evaporated. 1-Aminoisoquinoline-7-carboxylic acid (180mg), HATU (380mg, 1mmol.) and DIPEA (350μl, 2mmol.) dissolved in DMF (5ml) and stirred for 10 mins. This mixture was then added to the above product together with DBU (100μl). The mixture was then stirred
20 overnight. The mixture was then taken up into ethylacetate (50ml) and washed with water, sodium carbonate solution and water. The ethylacetate was then evaporated and the crude product purified by preparative hplc.

25 ¹H nmr (CD₃CN) 9.10 (1H, s); 8.42 (1H, d); 7.93 (1H, d); 7.65 (2H, d); 7.35 (1H,d); 7.30 (2H, m); 7.20 (1H,d); 7.00 (4H, m); 6.25 (1H, s); 4.25 (2H, m); 2.20 (3H, m). MS TOF 427 (M+1⁺). Hplc (Jupiter5 C18, Gradient 3,
30 water/acetonitrile/TFA) rt 11.48 min.

Example 85

35 **1-Amino-3,4-dihydroisoquinolin-7-oyl-D-cyclohexylglycine-1-S-cyclohexylethylamide**

A vigorously stirred solution of 1-Aminoisoquinolin-7-oyl-D-cyclohexylglycine-1-S-cyclohexylethylamide TFA

- 66 -

salt (100mg, 0.40 mmol) (see examples for Method 4) in 50%, 50% HCl/ethanol (10ml), containing platinum oxide (20mg) was treated with hydrogen gas at atmospheric pressure overnight. The reaction mixture was filtered through celite, concentrated and purified by preparative HPLC to yield the title compound as a white solid.

¹H nmr (DMSO) 8.40 (1H,s); 8.10 (1H,d); 7.55 (1H, d); 4.35 (1H, d); 3.70 (1H,m); 3.60 (2H,m); 3.10 (2H,m); 1.95-1.60 (broad 11H,m); 1.35-0.90 (broad 11H,m); 1.05 (3H,d); MS TOF 425 (M+1+). Hplc (Magellan C8, Gradient 3, water/acetonitrile/TFA) rt 11.42 min.

Example 86

3-([R]-1-phenyl-1-aminomethyl)-5-benzyl-2H-1,2,4-triazole

EDC (1.6g) was dissolved in DMF (20ml.) and treated with HOAt (1.16g) in DMF (10ml.) and stirred for 10 mins. DIPEA (1.46ml.) was then added and the stirring continued for a further 15 mins. Boc-D-phenylglycine (2g) in DMF (10ml.) was then added dropwise and stirring continued for a further 25mins. 1M Hydrazine in THF (84ml.) was then added and the mixture stirred over night. The solvents were then evaporated and the residue treated with water (100ml.), basified with 2M sodium hydroxide solution and extracted with ethylacetate. The combined extracts were washed with water and brine, dried with magnesium sulphate, filtered and evaporated to give a yellow solid (1.38g).

¹H nmr (CDCl₃) 7.20 (5H,s); 5.55 (1H,s); 1.25 (9H,s).

The above product (0.3g, 1.13mmol.) was dissolved in ethanol (6ml.) and added dropwise to a solution of benzyl ethyl imidate hydrochloride in ethanol (3ml.) at room temperature. The mixture was stirred for 30 mins.

- 67 -

The solvent was then evaporated and the residue treated with dilute sodium bicarbonate solution and extracted with ethylacetate. The combined extracts were washed with brine, dried over magnesium sulphate, filtered and
5 evaporated to give a yellow gum. (0.43g).

^1H nmr (CDCl_3) 7.30 (10H,m); 5.80 (1H,s); 3.50 (2H,m);
1.45 (9H,s).

10 The above crude product (0.43g, 1.12 mmol.) was dissolved in xylene (60ml.) with para toluenesulphonic acid (cat.) and heated to 160°C for 1 day. The xylene was removed under vacuo and the triazole (0.35g) used without further purification.

15 ^1H nmr (CDCl_3) 7.20 (10H,m); 5.70 (1H,s); 4.00 (2H,m);
1.30 (9H,s).

Coupling of this triazole intermediate to 1-
20 aminoisoquinoline-7-carboxylic acid can be carried out using the standard method Method 4.

Example 87

2-(1-Phenyl-1-aminomethyl)-4-phenylthiazole

25 To a solution of Boc-D-phenylglycine (875mg 3.5mmole) in a 1:1 mixture of DMF and DCM (40ml) was added 1-hydroxybenzotriazole (520mg 1.1equiv.) and DIPCI (602 μ l 1.1equiv.) and the mixture stirred for 30min at rt.
30 Ammonia gas was bubbled in and the mixture left overnight before diluting with ethyl acetate and washing with 10% hydrochloric acid and sat. sodium bicarbonate. The organic solution was dried (magnesium sulphate) and evaporated. Flash chromatography (silica gel
35 DCM/ethylacetate 0-50%) gave the amide 770mg 86%.
To a solution of Boc-D-phenylglycine amide (740mg 2.96mmole) in THF 25ml was added Lawesson's reagent

- 68 -

(1.2g) and the mixture stirred overnight. The solvent was evaporated off under reduced pressure and the residue purified by flash chromatography (silica gel - hexane/ethyl acetate 10 to 30%) to give the thioamide
5 671mg.

The thioamide (650mg) was dissolved in acetone (20ml) and phenacyl bromide (486mg 1 equiv) stirred for 30min and then diluted with chloroform/aqueous sod.
10 bicarbonate (20ml each), separated, dried (magnesium sulphate) and evaporated. The residue was dissolved in DCM (20ml) and treated with pyridine (350µl) and trifluoroacetic anhydride (360µl). After 150min the solvent was removed under reduced pressure and the
15 residues redissolved in DCM and washed with sat. sod. bicarbonate. Purification by flash chromatography (silica gel - hexane/ethyl acetate 10 to 30%) gave the thiazole intermediate 687mg.

20 Deprotection of the amine was carried out using 50% TFA in DCM (30min), evaporation of solvent under reduced pressure, dissolving in DCM, washing with sat sod. bicarbonate, drying (magnesium sulphate) and evaporation to yield the product.

25

¹H nmr (CDCl₃) 7.80 (1H,d); 7.25 (10H,m); 5.95 (1H,s).

Coupling of this thiazole intermediate to 1-aminoisoquinoline-7-carboxylic acid can be carried out
30 using the standard method Method 4.

Example 88

1-Aminoisoquinoline-7-carboxylic acid

35 A 1L flask was equipped with magnetic stirring bar, cooling bath (liquid nitrogen), stopcock connected to an ammonia cylinder and a vacuum system. The flask was

- 69 -

charged with FeCl_3 (0.1g) and evacuated. Ammonium gas was condensed to give liquid ammonium (600ml). Potassium (19.50g 0.50mole) was added in small pieces (each about 0.5g) under an argon atmosphere. The solution initially became blue but became a grey suspension after heating at reflux for 30 min. Into the potassium amide/liquid ammonia suspension was added in small portions isoquinoline-7-carboxylic acid {ref F.T.Tyson, JACS, 1939, 61, 183} (17.0g 0.10mole). The free acid initially dissolved but the formed potassium salt soon precipitated as fine particles to give a suspension which was heated at reflux for 6 h. After careful addition of tetraethylethylene diamine (TEEDA) (86g 0.50mole), toluene (170ml) and 18-crown-6 (1g) the ammonia was allowed to evaporate slowly over 40 h to give a new suspension of the isoquinoline-7-carboxylic acid potassium salt. The reaction mixture was heated to 87°C for 48 h. A sample was removed and analysed by nmr which indicated that there was still some unreacted starting material present. Ammonia was bubbled through the reaction mixture for 2 h and nmr analysis then revealed that no starting material remained. The reaction was then cooled to -16°C, ice (200g) was added to destroy excess potassium amide and all solvents evaporated at 76°C and 7mmHg and then at 65°C and 0.7mmHg to give a powder which was dissolved in water (1.2L) and filtered through Celite to give a clear solution. This was acidified to pH 7 by addition of acetic acid (34g) and allowed to stand overnight. The precipitate was collected by filtration, washed with water (50ml x 2) and dried by azeotroping with ethanol (50ml x 2) at 50°C and 7mmHg then at 50°C and 0.7mmHg to give the crude product (17.6g 94%) as an off-white powder. The crude product (17.6g) was dissolved in water (4L), and concentrated HCl was added to give a pH of 1.30. The solution was stirred for 1 h, and filtered through Celite to give a clear solution to which was added

- 70 -

concentration ammonia solution to give a pH of 5.07. After stirring for 2 h and standing overnight the precipitate was filtered, washed with water (50ml x2) and dried by azeotropeing with ethanol (50ml x 2) at 50°C and 7mmHg, and then at 50°C and 0.7mmHg to give title compound (15.5g 88%) as a pale brown solid.

¹H nmr (DMSO) 9.20 (1H,s); 8.40 (1H,d); 8.10 (1H,d); 7.85 (1H,d); 7.30 (1H,d).

Example 89

Ethyl 1-aminoisoquinoline-7-carboxylate hydrochloride

To a suspension of 1-aminoisoquinoline-7-carboxylic acid TFA salt (6g, 20mmol) in anhydrous ethanol (150mL) was added thionyl chloride (7.7 mL, 10 eq.) slowly at 00C and the reaction mixture was then refluxed for 5 hours. The volatiles were removed under vacuo and the resultant solid treated with diethyl ether and filtered to give the title compound as a brown solid.

¹H nmr (D₂O) 8.40 (1H,s); 8.05 (1H,d); 7.60 (1H,d); 7.40 (1H,d); 6.95 (1H,d); 4.30 (2H,q); 1.35 (3H,t).

Example 90

Ethyl 1-benzylaminoisoquinoline-7-carboxylate trifluoroacetate

To a suspension of ethyl 1-aminoisoquinolinecarboxylate HCl salt (126mg, 0.5 mmol), and potassium carbonate (152mg, 1.1 mmol) in anhydrous DMF was added benzyl bromide (65μL, 0.55 mmol) and the reaction mixture left to stir at room temperature overnight. Purification by preparative HPLC gave the title compound as a white solid.

¹H nmr (CD₃OH) 9.05 (1H,s); 8.30 (1H,d); 7.85 (1H,d);

- 71 -

7.55 (1H,d); 7.20 (5H,m); 7.05 (1H,d); 5.35 (2H,s); 4.30 (2H,q); 1.25 (3H,t).

Example 91**5 Ethyl 1-amino-3,4-dihydroisoquinoline-7-carboxylate trifluoroacetate**

A vigorously stirred solution of ethyl 1-aminoisoquinoline-7-carboxylate (100mg, 0.40 mmol) in
10 50% hydrochloric acid solution (10ml), containing platinum oxide (20mg) was treated with hydrogen gas at atmospheric pressure overnight. The reaction mixture was filtered through celite, concentrated and purified by preparative HPLC to yield the title compound as a
15 white solid.

¹H nmr (CD₃CN) 8.40 (1H,s); 8.15 (1H,d); 7.45 (1H,d); 4.30 (2H,q); 3.45 (2H,m); 3.05 (2H,m); 1.35 (3H,t).

20 Example 92**1-Amino-7-hydroxymethylisoquinoline**

Ethyl 1-aminoisoquinolinecarboxylate hydrochloride (1g) was partitioned between ethylacetate and 2M sodium
25 hydroxide solution. The ethylacetate layer was washed with brine, dried over magnesium sulphate, filtered and evaporated to dryness. The residue was dissolved in dry THF (10ml.), cooled to -5°C and a 1M solution of diisobutylaluminium hydride (12mls.) added dropwise.
30 The mixture was stirred at 0°C for 2 hrs. then at room temperature for a further 2 hrs. Ethyl acetate (50ml.) and 2M sodium hydroxide solution (5ml.) were then added. The ethylacetate solution was then separated, washed with brine, dried over magnesium sulphate, filtered and
35 evaporated to give the product as a brown solid.

¹H nmr (CD₃CN) 7.80 (1H,d); 7.70 (1H,s); 7.60 (1H,d);

- 72 -

7.50 (1H,d); 6.90 (1H,d).

Example 93**1-Amino-7-bromomethylisoquinoline**

5

1-Amino-7-hydroxymethylisoquinoline (0.2g) was dissolved in dry THF (10ml.) and phosphorous tribromide (0.03 ml.) added in one portion. A white precipitate was seen to form and the suspension was stirred for 1 hr. at room temperature. The mixture was evaporated to dryness and triturated with diethylether to give the product.

10

¹H nmr (CD₃CN) 8.20 (1H,s); 7.80 (1H,d); 7.60 (1H,d); 7.35 (1H,d); 7.00 (1H,d).

15

Assay protocols**Enzyme Inhibition assays:**

Enzyme assays were carried out at room temperature in 0.1M phosphate buffer, pH7.4 according to the method of Tapparelli et al (J. Biol. Chem. 1993,268,4734-4741). Purified human factor Xa, trypsin, thrombin and plasmin were purchased from Alexis Corporation, Nottingham, UK. Urokinase was purchased from Calbiochem, Nottingham, UK. Chromogenic substrates for these enzymes; pefachrome-FXA, pefachrome-TRY, pefachrome-TH, pefachrome-PL and pefachrome-UK were purchased from Pentapharm AG, Basel, Switzerland. Product (p-nitroaniline) was quantified by adsorption at 405nm in 96 well microplates using a Dynatech MR5000 reader (Dynex Ltd, Billingshurst, UK). Km and Ki were calculated using SAS PROC NLIN (SAS Institute, Cary, NC, USA, Release 6.11) K_m values were determined as 100.9μM for factor Xa/pefachrome-FXA and 81.6μM for trypsin/pefachrome-TRY. Inhibitor stock solutions were prepared at 40mM in Me₂SO and tested at 500μM, 50μM and 5μM. Accuracy of Ki measurements was

35

- 73 -

confirmed by comparison with K_i values of known inhibitors of factor Xa and trypsin.

In agreement with published data, benzamidine inhibited factor Xa, trypsin, thrombin, plasmin and urokinase with K_i values of 155 μ M, 21 μ M, 330nM, 200nM and 100nM respectively. NAPAP inhibited thrombin with a K_i value of 3nM. Compounds of the invention were found to have activity in these assays. By way of example the inhibitors described in Example 75 demonstrated a K_i of 8nM and Example 8 demonstrated a K_i of 70nM when tested against Factor Xa.

Antithrombotic Activity

The test material (Factor Xa inhibitor) was administered either intravenously, intraperitoneally or orally to a group of rats for experiment. A second group received vehicle (saline) only as a control, and a third group of animals received a standard antithrombotic (subcutaneous lmw heparin) as a positive control.

To perform the experiment male Sprague-Dawley rats (250-400g in weight) were anaesthetised by the inhalation of isoflurane with the addition of oxygen and nitrous oxide. The left or right femoral vein was carefully exposed and isolated from the femoral artery and saphenous nerve. Following removal of connective tissue a cannula containing physiological saline was inserted into the femoral vein.

A segment of each of the left and right jugular vein was exposed and separated from the surrounding connective tissue. Each segment consisted of the section of vein between the exit point from the thorax to the vessel's first major junction.

- 74 -

At the desired interval following the administration of the test material or vehicle a bolus injection of 'deactivated' human serum (1.32 ml.kg^{-1}) was administered over less than 30 seconds, via the femoral vein cannula.

5 Two minutes following the thrombus challenge both the jugular vein segments were ligatured at both ends and left *in situ* for thrombi to form.

After 10 min both jugular segments were carefully excised, and placed in a petri dish containing 0.9% saline. A blood sample (1.8 ml blood + 0.2 ml 3.8% sodium citrate) was obtained by cardiac puncture and the animal sacrificed by an overdose of Expiral (sodium pentobarbitone) administered intravenously via the

10 femoral vein cannula or by cardiac puncture. The 2 segments of jugular vein were carefully dissected longitudinally along one surface to expose the lumen and dispel the vessel contents into the saline. The tissues were examined for the presence of any developed thrombi

15 and scored accordingly.

20

Thrombus score:

- 0 = No thrombus
- 25 1 = One or several small thrombi
- 2 = Several larger thrombi
- 3 = Large thrombus occluding the vessel

Compounds of the invention were found to have significant antithrombotic activity in these assays.

30

Partial Thromboplastin Time Test Protocol

Venous blood was collected into 3.2% (0.109M) trisodium citrate vacutainer tubes at 1 volume of anticoagulant to

35 nine volumes of blood.

- 75 -

The blood cells were separated by centrifugation at 700g for ten minutes to yield plasma, which was frozen at 70°C until required.

- 5 To perform the test, 100 μ l of plasma was pipetted into in a glass test tube, 1 μ l of test compound in DMSO was added, and allowed to warm to 37° over two minutes.

- 10 100 μ l of warm (37°) Manchester (tissue thromboplasin) reagent (Helena Biosciences, UK) was added, allowed to equilibrate for sixty seconds.

- 15 100 μ l of warm (37°) 25mM calcium chloride solution was added to initiate clotting.

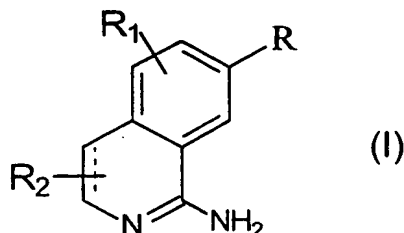
- The test tube was tilted three times through a 90° angle every five seconds to mix the reagents and the time to clot formation recorded.

- 20 Data from a series of observations and test compound concentrations are analysed by a SAS statistical analysis program and a CT2 (Concentration required to double clotting time) for each compound is generated.

- 25 Compounds of the invention were found to significantly elongate the partial thromboplastin time.

Claims:

1. A serine protease inhibitor compound of formula I



(where R_1 is hydrogen, halo, cyano, nitro or hydroxyl, amino, alkoxy, alkyl, aminoalkyl, hydroxyalkyl, thiol, alkylthio, aminosulphonyl, alkoxyalkyl, alkoxycarbonyl, acyloxymethoxycarbonyl or alkylamino optionally substituted by hydroxy, alkylamino, alkoxy, oxo, aryl, cycloalkyl, amino, halo, cyano, nitro, thiol, alkylthio, alkylsulphonyl, alkylsulphenyl, alkylsulphonamido, alkylaminosulphonyl, haloalkoxy and haloalkyl;

15

20

R_2 is hydrogen, halo, methyl, amino, hydroxy or oxo; and

R is $X-X-Y(R_7)-L-L_p(D)_n$

25 (wherein each X independently is a C, N, O or S atom or a CO, CR_1 , $C(R_1)_2$ or NR_1 group, at least one X being C, CO, CR_1 or a $C(R_1)_2$ group;

Y (the α -atom) is a nitrogen atom or a CR_1 group or Y and L taken together form a cyclic group;

30

R_7 is a lipophilic group selected from alkyl, alkenyl, mono- or bi-cycloalkyl, aryl, heteroaryl, mono- or bicycloalkylalkyl, mono- or bicycloalkylalkenyl, aralkyl, heteroaryl-alkyl, arylalkenyl, heteroarylalkenyl all optionally substituted by a group R_1 ;

35

L is an organic linker group containing 1 to 5

- 77 -

backbone atoms selected from C, N, O and S, or a branched alkyl or cyclic group;

Lp is a lipophilic organic group selected from alkyl, heterocyclic, alkenyl, alkaryl, cycloalkyl, polycycloalkyl, cycloalkenyl, aryl, aralkyl or haloalkyl group or a combination of two or more such groups optionally substituted by one or more of oxa, thia, aza or R₁ groups;

D is a hydrogen bond donor group; and n is 0, 1 or 2) or a physiologically tolerable salt thereof.

2. A compound as claimed in claim 1 wherein the double bond is present between the 3 and 4 positions of the fused ring.

3. A compound as claimed in claim 1 or 2 wherein R₁ represents hydrogen, hydroxy, amino or alkyl.

4. A compound as claimed in any one of claims 1 to 3 wherein R₁ is on the 6-position of the fused ring.

5. A compound as claimed in any one of claims 1 to 4 wherein R₂ is hydrogen.

6. A compounds as claimed in anyone of claims 1 to 5 wherein the linker X-X is -CH=CH-, -CONH-, -CONR₁-, -NH-CO-, -NH-CH₂-, -CH₂-NH-, -CH₂O-, -OCH₂-, -COO-, -OC=O- or -CH₂CH₂-.

7. A compound as claimed in claim 6 wherein the X moiety nearest Y is NH.

8. A compound as claimed in claim 6 or 7 wherein the X moiety nearest the fused ring is CH₂ or CO.

- 78 -

9. A compound as claimed in claim 6 wherein X-X is
-CONH-.

10. A compound as claimed in anyone of claims 1 to 9
5 wherein Y is CH.

11. A compound as claimed in any one of claims 1 to 10
wherein R_7 represents an unsubstituted or R_1 -substituted
aryl, heteroaryl or cyclohexyl group.

10

12. A compound as claimed in claim 11 wherein R_7
represents phenyl or naphthyl.

13. A compounds as claimed in any one of claims 1 to 12
15 wherein the linker L represents CO , CH_2NH , $\text{CONR}_1(\text{CH}_2)_m$,
 $(\text{CH}_2)_m\text{N}(\text{R}_1)\text{CO}(\text{CH}_2)_m$, $(\text{CH}_2)_{m+2}$, $(\text{CH}_2)_m\text{CO}(\text{CH}_2)_m$, $(\text{CH}_2)_m\text{OC}=\text{O}$,
 $(\text{CH}_2)_m\text{O}$ or $\text{CH}=\text{CH}(\text{CH}_2)_m$ (where each m is independently 0 or
1).

14. A compound as claimed in claim 13 wherein the
20 linker L represents $\text{CONR}_1(\text{CH}_2)_m$, CO or $(\text{CH}_2)\text{NR}_1\text{CO}$ where m
is 0 or 1.

15. A compound as claimed in any one of claims 1 to 14
25 wherein said lipophilic group comprises a cycloalkyl,
azacycloalkyl, diazacycloalkyl, phenyl, naphthyl,
adamantyl, decaliny, tetrahydrodecalinyl, bicycloalkyl,
mono- or diazabicycloalkyl, mono- or bicyclo
heteroaromatic or a linear or branched alkyl, alkylene,
30 alkenyl or alkenylene group all optionally substituted
by oxa, aza, thia or one or more groups R_1 , or a
combination of at least two such groups linked by a
spiro linkage or a single or double bond or by $\text{C}=\text{O}$, O ,
 S , SO , SO_2 , CONR_1 , $\text{NR}_1\text{-CO-}$, NR_1 linkage.

35

16. A compound as claimed in claim 15 wherein the
lipophilic group is a methylcyclohexyl,

- 79 -

methyleyclohexylmethyl, methylphenylmethyl, phenylethyl, benzylpiperidinyl, benzoylpiperidinyl, bispiperidinyl or phenylpiperazinyl.

5 17. A compound as claimed in any one of claims 1 to 16 wherein the hydrogen bond donor group is attached to the lipophilic group.

10 18. A compound as claimed in claim 17 wherein nitrogen or oxygen is the hydrogen bond donor atom.

15 19. A compound as claimed in claim 18 wherein the hydrogen bond donor is formed from a hydroxyl group, a primary, secondary or tertiary amine, or a primary or secondary imine group as part of an amidine or guanidine or a saturated or unsaturated heterocyclic group containing 5 to 7 ring atoms and having a ring nitrogen.

20 20. A compound as claimed in any one of claims 1 to 19 wherein Y is carbon and preferably has the conformation that would result from construction from a D- α -aminoacid $\text{NH}_2\text{-CR}_1(\text{R}_7)\text{-COOH}$.

25 21. A compound as claimed in any one of claims 1 to 20 wherein the serine protease to be inhibited is Factor Xa.

30 22. A pharmaceutical composition comprising a serine protease inhibitor as claimed in any one of claims 1 to 21 together with at least one pharmaceutically acceptable carrier or excipient.

35 23. A serine protease inhibitor as claimed in any one of claims 1 to 22 for use in combatting a condition responsive to said inhibitor.

- 80 -

24. Use of a serine protease inhibitor as claimed in any one of claims 1 to 21 for the manufacture of a medicament for use in a method of treatment of the human or non-human animal body to combat a condition responsive to said inhibitor.

25. Use as claimed in claim 24 wherein said condition is a thrombotic disease, asthma, emphysema, cirrhosis, arthritis, carcinoma, melanoma, restenosis, atheroma, trauma, shock and reperfusion injury.

26. A method of treatment of the human or non-human animal body to combat a condition responsive to a serine protease inhibitor, said method comprising administering to said body an effective amount of a serine protease inhibitor as claimed in any one of claims 1 to 21.

27. The compounds 1-Aminoisoquinolin-7-oyl-D-phenylglycyl-D-2-naphthylalaninylproline, 1-aminoisoquinolin-7-oyl-D-phenylglycine-4-(4'-pyridyl) piperidinamide, 1-Aminoisoquinolin-7-oyl-D-phenylglycine-4-methoxybenzylamide, 1-Aminoisoquinolin-7-oyl-D-phenylglycine-4-(4'-hydroxyphenyl) piperazinamide, 1-Aminoisoquinolin-7-oyl-D-phenylglycine-4-(2,4-difluorophenyl) piperazinamide, 1-Aminoisoquinolin-7-oyl-D-cyclohexylglycine-4-(4'-pyridyl) piperazinamide, 1-Aminoisoquinolin-7-oyl-D,L-1-naphthylglycine-4,4-bipiperidinamide, 2(R)-{[N-(1-Aminoisoquinoline)-7-oyl]amino}-2-phenylethyl-4-methylbenzamide and 1-Aminoisoquinolin-7-oyl-D-phenylglycine-4,4'-(1'-methyl)bipiperidinamide.

28. 1-amino-7-carbonyl-isoquinolines and 3,4-dihydro-1-amino-7-carbonyl-isoquinolines

29. A compound as claimed in claim 28 having an amino-carbonyl substituent at the 7-position.

- 81 -

30. A compound as claimed in either one of claims 28 and 29 having an amino group at the 6-position.

5 31. 1-Aminoisoquinoline-7-carboxylic acids and salts, esters and amides thereof.

32. A compound as claimed in claim 31 having an amino group at the 6-position.

INTERNATIONAL SEARCH REPORT

International Application No.

PCT/GB 98/02600

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C07K5/06 C07K5/08 A61K38/05 A61K38/06 A61K38/07
 A61K31/47 C07D217/22 C07D401/12 C07D401/14 C07D405/12
 C07D409/14 //(C07D401/12,217:00,207:00),(C07D401/12,217:00,

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C07K A61K C07D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	CHEMICAL ABSTRACTS, vol. 103, no. 25, 23 December 1985 Columbus, Ohio, US; abstract no. 209699z, XP002083330 see abstract & R. FERRONI ET AL.: FARMACO, ED. SCI., vol. 40, no. 10, 1985, pages 717-729, ---	1-32
A	CHEMICAL ABSTRACTS, vol. 108, no. 7, 15 February 1988 Columbus, Ohio, US; abstract no. 48724q, XP002083331 see abstract & R. FERRONI ET AL.: FARMACO, ED. SCI., vol. 42, no. 10, 1987, pages 709-715, --- -/--	1-32

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents:

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "&" document member of the same patent family

Date of the actual completion of the international search

6 November 1998

Date of mailing of the international search report

24/11/1998

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
 NL - 2280 HV Rijswijk
 Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
 Fax: (+31-70) 340-3016

Authorized officer

Frelon, D

INTERNATIONAL SEARCH REPORT

Patent Application No
PCT/GB 98/02600

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 211:00)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	CHEMICAL ABSTRACTS, vol. 71, no. 7, 18 August 1969 Columbus, Ohio, US; abstract no. 28982m, XP002083332 see abstract & B.R. BAKER ET AL.: JOURNAL OF MEDICINAL CHEMISTRY, vol. 12, no. 3, 1969, pages 408-414, WASHINGTON US	1-32
A	EP 0 672 658 A (LILLY CO ELI) 20 September 1995 see examples 16,24,25,27,31,33,36,37,40,41 --- -/--	1-32

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "&" document member of the same patent family

Date of the actual completion of the international search

6 November 1998

Date of mailing of the international search report

Name and mailing address of the ISA
European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040. Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Frelon, D

INTERNATIONAL SEARCH REPORT

International Application No.

PCT/GB 98/02600

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 97 24135 A (LU TIANBAO ;TOMCZUK BRUCE E (US); ILLIG CARL (US); SOLL RICHARD M) 10 July 1997 see examples 6,7,15,17 ---	1-32
A	WO 95 13274 A (PFIZER LTD ;PFIZER RES & DEV (IE); PFIZER (US); DANILEWICZ JOHN CH) 18 May 1995 see claims ---	1-32
A	WO 92 08709 A (PENTAPHARM AG) 29 May 1992 see claims ---	1-32
A	CHEMICAL ABSTRACTS, vol. 101, no. 1, 2 July 1984 Columbus, Ohio, US; abstract no. 6764c, XP002083333 see abstract & H. VIEWEG ET AL.: PHARMAZIE, vol. 38, no. 12, 1983, pages 818-820, BERLIN DE -----	1-32

INTERNATIONAL SEARCH REPORT

International application No.

PCT/GB 98/02600

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 26
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claim 26
is directed to a method of treatment of the human/animal
body, the search has been carried out and based on the alleged
effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such
an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all
searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment
of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report
covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is
restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/GB 98/02600

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0672658 A	20-09-1995	AU 684918 B AU 1975295 A BR 9506979 A CA 2183464 A CN 1147205 A CZ 9602584 A FI 963451 A HU 76330 A JP 9509937 T NO 963684 A NZ 282588 A PL 320637 A WO 9523609 A US 5705487 A US 5726159 A US 5707966 A	08-01-1998 18-09-1995 18-11-1997 09-08-1995 09-04-1997 11-06-1997 03-09-1996 28-08-1997 07-10-1997 28-10-1996 19-12-1997 13-10-1997 08-09-1995 06-01-1998 10-03-1998 13-01-1998
WO 9724135 A	10-07-1997	AU 1432397 A NO 982987 A	28-07-1997 28-08-1998
WO 9513274 A	18-05-1995	AT 155464 T AU 680565 B AU 8104994 A CA 2176037 A CN 1134148 A CZ 9601322 A DE 69404325 D DE 69404325 T DK 728132 T EP 0728132 A ES 2105868 T FI 961939 A GR 3024228 T HU 76268 A JP 2739776 B JP 9500391 T NO 961841 A NZ 275798 A PL 314237 A US 5750520 A	15-08-1997 31-07-1997 29-05-1995 18-05-1995 23-10-1996 14-08-1996 21-08-1997 30-10-1997 01-09-1997 28-08-1996 16-10-1997 07-05-1996 31-10-1997 28-07-1997 15-04-1998 14-01-1997 07-05-1996 22-09-1997 02-09-1996 12-05-1998

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/GB 98/02600

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9513274 A		ZA 9408772 A	07-05-1996
WO 9208709 A	29-05-1992	AU 8868991 A	11-06-1992
		CA 2073776 A	16-05-1992
		EP 0511347 A	04-11-1992
		JP 5503300 T	03-06-1993
		US 5518735 A	21-05-1996

THIS PAGE BLANK (USPTO)